The Use and Quality Control of Leukocyte-Depleted Cell Concentrates

Although secondary alloimmunization against HLA class I antigens cannot be prevented by the removal of leukocytes from cell concentrates, there is ample evidence that primary immunization is nearly always prevented if all transfused concentrates contain $<5 \times 10^6$ leukocytes. Furthermore, removal of leukocytes may prevent the transmission of some infectious agents, e.g. CMV and HTLV I/II. Although platelet concentrates (PC) containing $<1 \times 10^6$ leukocytes can now be prepared in certain apheresis machines, filtration is still usually applied to reduce the number of residual leukocytes to the desired low numbers. Several aspects concerning the filtration of red cell concentrates (RCC) and PC are not standardized and it seemed of interest to obtain further information on the subject in an International Forum.

To obtain such information the questions listed below were sent to 14 experts in the field. Contributions to the forum were received from 9 of them.

1. Do you use leukocyte-depleted RCC and PC routinely or only with special indications and if so, which are these indications?
2. Which filters are used for RCC and PC?
3. Is filtration performed in the laboratory or at the bedside?
4. What is your quality assurance procedure for the filtered concentrates?
5. At what time after the blood has been taken is filtration performed and what is the reason for the chosen time?
6. How do you think a new filter (or apheresis system) should be validated to ensure performance comparable to that claimed by the manufacturer?
7. Do you use other methods than filtration to make leukocyte-depleted products routinely? If yes, please specify and describe in respect to questions 4, 5 and 6.
8. Which method of leukocyte counting in the leukocyte-depleted product is used and what is the precision of this assay?

From the answers the following observations and conclusions can be made. In none of the centers are RCC routinely leukocyte-depleted and neither are PC except in one center (see Massa and Naegelen), the reasons being the costs of filtration and/or the fact that leukocyte-depleted concentrates are really indicated for only some categories of patients. The answers confirm the three main indications for leukocyte-depleted concentrates: (1) the prevention of alloimmunization against HLA class I antigens (except in 1 center, see Heddle), (2) to prevent the transmission of CMV and (3) to prevent serious febrile transfusion reactions in alloimmunized patients who still develop such reactions when givenuffy-coat-free RCC.

(1) This concerns patients who are or may become candidates for bone marrow, stem cell or organ transplantation (unless nonleukodepleted blood is part of an immunosuppressive protocol) and, in some centers, patients expected to need many transfusions or transfusions over a long period of time, e.g. patients with hematological malignancies, aplastic anemia or an inherited form of anemia such as thalassemia. Additional indications are intrauterine transfusions and transfusions to infants to avoid possible short-term or long-term immunosuppressive effects in premature infants.

(2) To avoid transmission of CMV, concentrates are leukocyte-depleted for intrauterine transfusions, for transfusions to infants, pregnant women and for patients with immunodeficiency. Interestingly, hospitals in San Francisco with a large population of AIDS patients have converted to 100% leukocyte-depleted products because of the possibility that viral infections in the recipients may otherwise be reactivated.

For information concerning the filters used, we refer to the answers from the experts. It seems important that there is an increasing interest in filtering whole blood and that some filters are being or have been validated for this purpose. In the majority of the centers (5 of 9) filtration is exclusively performed in the laboratory because proper quality control is considered to be impossible at the bedside. In one of these centers bedside filtration was practised between 1990 and 1995 but has then been stopped (see Myllylä).

In another center there is a tendency to replace bedside filtration by filtration in the laboratory, but for a different reason, i.e. the growing conviction that leukocytes should be removed early, i.e. within 24 h after blood collection (see Myllyö and see below).

The quality control described in the answers refers almost exclusively to concentrates filtered in the laboratory. On the whole, residual leukocytes are counted in about 1–5% of filtered concentrates. These percentages are based on the experience obtained in validating the filters or on the advise of the Council of Europe [1]. In one center it is advocated to validate a new filter by counting leukocytes in at least 200 filtered concentrates. If there are no failures quality checks are reduced to 1% (Pietersz and Dekker).

Quality control at the bedside is a difficult issue. In one center because reliable postfiltration samples from concentrates filtered at the bedside are not available, the quality assurance of bedside filtration relies on the qualification and training of nurses (see Rebulla et al.). Obviously, the question of bedside filtration is important because of the opinions expressed by several experts and the observation that transfusion of concentrates
filtered at the bedside results in only a small, statistically insignificant reduction in the incidence of HLA class I antibodies [2].

RCC or whole blood are filtered within 24 h in some an not later than 3–5 days after the blood is taken in most other centers. In one center buffycoat-free RCC are prepared from units of whole blood after maximally 18 h of storage at room temperature. The RCC are then stored overnight at 4°C (minimum 16 h) and then filtered. The cold storage results in a more effective removal of leukocytes and it is essential for meeting requirements in Finland (see Myllylä). PC are filtered within 24 h in nearly all centers. The reasons for early filtration are the usual: removal of intact leukocytes containing bacteria, no leukocyte fragments, a decreased concentration of cytokines and filtration as part of a routine preparation process is practical.

Strict validation is carried out in all centers. For details we refer to the various contributions. A draft of a standard user validation approach is being developed by the BEST subcommittee of the ISBT and is expected to be ready by 1998 (see Heaton and Adams). It is stressed by several experts that in addition to validating the filtration procedure itself, it is also necessary to validate the function of the cells after preparation and storage.

In most centers filtration is the only method of leukocyte depletion. In East Anglia preparation of leukocyte-depleted PC by COBE LRS apheresis technology will be implemented this year (1997) (see Williamson). This technique is also used in another center (see Heddle and Adams).

Residual leukocytes are counted in the Nageotte chamber in all centers except in the center, where PCR is used to count the number of residual leukocytes in filtered RCC (see Adams and Heaton).

All in all, the process of leukocyte depletion of cell concentrates by filtration seems to be a fairly well-standardized procedure. There is a consensus of opinion that leukocyte depletion should not be done routinely but should be restricted to a number of well-defined indications.

References


Ludo Myllylä

Question 1

Leukocyte-depleted red cell and platelet concentrates are used on special indications. The major indication is the prevention of HLA alloimmunization in patients that may require multiple transfusions, transplant candidates and transplanted patients. Additional indications are the prevention of febrile non-hemolytic transfusion reactions in patients with a history of febrile reactions to at least two transfusions of blood components and the prevention of the transmission of cytomegalovirus if a cytomegalovirus antibody-negative blood component is indicated but not readily available.

Question 2

Red cells are filtered using BPF4-B filters (Pall, Glenn Cove, N.Y.) or Sepacell R-500 filters (Baxter). Platelets are filtered using PL-50, PL-100 filters or autostop filters (Pall). Units of leukocyte-depleted apheresis platelets are prepared by a SPECTRA (Cobe BCT, Lakewood, Colo.) apheresis machine using the LRS system.

Question 3

The majority of the filtrations is performed at the bedside. This decision was taken several years ago for practical reasons: bedside filtration is simple, it reduces the cost (no additional transportation or laboratory work load) and the loss of filtered products because of a short storage time. However, there is a growing evidence that leukocytes should be removed early, i.e. within 24 h after blood collection from blood components: improved removal of leukocytes, removal before leukocyte fragmentation, prevention of increased cytokine plasma levels, removal of bacteria and improved quality assurance [1, 2]. Furthermore, systems such as in-line filters and sterile docking devices are available allowing the removal of leukocytes in a closed system and consequently a normal storage period of the leukocyte-depleted blood components. For these reasons there is a tendency to move from bedside filtration to laboratory filtration. In the same context a discussion has started on the routine use of leukocyte-depleted platelet concentrates for all patients because it is that type of platelet concentrate most patients require. It would simplify the procedure of preparation, the inventory, the issue and the transfusion of platelets and increase the quality of the products.

Question 4

Residual leukocytes should be below 1 × 10^5 per unit of red cell or platelets (adult dose: 2.5–4 × 10^6 platelets). This parameter is controlled in 1% of leukocyte-depleted red cells and 5% of leukocyte-depleted platelets filtered in the laboratory and 90% of the units must contain values below the maximum leukocyte load [3]. In the remaining 10% the leukocyte content should never exceed 5 × 10^4 per unit. A leukocyte-depleted random donor platelet concentrate should contain at least 0.5 × 10^11 platelets per single unit equivalent in order to avoid exposure of the patient to an unnecessary high number of donors.

Question 5

When filtration is performed in the laboratory we have chosen to remove leukocytes from red cells and platelets within 24 h of donation. In addition to the reasons given earlier, filtration performed as part of a routine component preparation process is also an element of practical (and economical) importance.

Question 6

First of all the manufacturer should clearly specify the leukocyte removal capacity of the device expressed as a total number of leukocytes removed early, i.e. within 24 h after blood collection from blood components: improved removal of leukocytes, removal before leukocyte fragmentation, prevention of increased cytokine plasma levels, removal of bacteria and improved quality assurance [1, 2]. Furthermore, systems such as in-line filters and sterile docking devices are available allowing the removal of leukocytes in a closed system and consequently a normal storage period of the leukocyte-depleted blood components. For these reasons there is a tendency to move from bedside filtration to laboratory filtration. In the same context a discussion has started on the routine use of leukocyte-deplet
tation as described by Dumont et al. [4] is a simple method to assess process capability. However, as a consequence of advances in the development of new devices the residual leukocyte content continues to decrease and the accurate and precise counting of this number will be more and more difficult. In respect of this it also would be very important to dispose of a set of standards of low leukocyte content.

Question 7
Apart from the use of the leukocyte-reducing system to prepare leukocyte-depleted apheresis platelets, no methods other than filtration to make leukocyte-depleted products are used routinely.

Question 8
The samples of red cells and platelets are diluted in Türk’s solution (Merck, Belgolabo, Belgium; red cells, dilution 1:10; platelets, dilution 1:5) and leukocytes are counted in the Nageotte Hemocytometer with a large volume chamber (50 µl). Precision of counting leukocytes in red cells is 9% (2 leukocytes/µl) and 20% (1 leukocyte/µl).

Note Added in Proof
Since submitting my response to these questions, the Canadian Blood Agency has approved finding in Canada to pre-storage leukoreduce 100% of the platelet supply. This is scheduled to be implemented across Canada by February 1998. Red cell concentrates will not be pre-storage leukoreduced.

References
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Nancy M. Heddle

Question 1
Current data suggests that leukocyte-reduced blood products are useful in the following situations: to prevent febrile nonhemolytic transfusion reactions to red cells, to prevent HLA alloimmunization, and to prevent the transmission of cytomegalovirus (CMV). However, the role of leukocyte reduction in immune suppression is still controversial.

On the basis of this assessment, leukocytreduced red cell and platelet products are not routinely used at our hospital. Leukocyte-reduced red cells are provided for patients with recurrent febrile nonhemolytic transfusion reactions (FNHTR). We remove the plasma (cytokines) from the platelet product as the primary intervention to prevent platelet reactions [1]. If a patient continues to react to plasma-reduced platelets, the platelet product is also leukocyte-reduced by filtration. Leukocyte reduction is being used in a clinical trial to evaluate three types of platelet products (plasma reduced, prestorage leukocyte-reduced random donor platelets, and LRS pheresis platelets (COBE BCT, Lakewood, Colo.), to determine which is associated with fewer adverse effects. Leukocyte reduction is not used to prevent alloimmunization, but would be used to prevent CMV transmission if serologically screened CMV-negative blood products were not available [2].

Question 2
Leukocyte reduction of red cells is usually performed by an inverted centrifugation technique as this procedure is effective in reducing the number of leukocytes to the critical threshold of 5 x 10^6 leukocytes per product [3]. Patients who continue to react to this product receive filtered red cells using a spin cool filtration technique using the SQ40S filter (Pall Biomedical, East Hills, N.Y.) [4].

In a recently completed clinical trial investigating FNHTR we used the LRF6 laboratory filter (Pall Biomedical, East Hills, N.Y.) to leukocyte-reduce platelets and found that 97% of products had residual leukocyte counts <5 x 10^6/product with only a 4.9% platelet loss. Because of the high level of cytokine accumulation in this poststorage leukocyte-reduced platelet product, FNHTR still occurred; hence, this approach is not used at our center.

Question 3
In the selected circumstances where filtered blood products are indicated, filtration is performed using a laboratory filter. Laboratory filtration provides a more standardized leukocyte-reduced product than bedside filtration and is more conducive to quality control.

Question 4
The leukocyte-reduced platelet products that are being used for patients participating in the clinical trial at our center are manufactured by the Canadian Red Cross Society, Hamilton Centre. The quality assurance process follows the recommendations published by the Biomedical Excellence for Safer Transfusion (BEST) Working Party of the International Society of Blood Transfusion (ISBT) [5]. These recommendations are a very practical and effective approach to quality assurance for leukocyte-reduced products.

Question 5
Red cell concentrates are filtered poststorage. At the present time, our blood supplier (Canadian Red Cross Society) is not licensed to prepare prestorage leukocyte-reduced red cells. In our current clinical trial investigating reactions with three different types of platelet products, two of the products are prestorage leukocyte-reduced (random donor platelets and LRS pheresis platelets). We anticipate that these products will have the lowest frequency of reactions as cytokines do not accumulate during storage and the product is leukocyte-reduced.

There are several hypothetical arguments which suggest that prestorage leukocyte reduction may be more effective than poststorage leukocyte reduction, i.e. some biological response modifiers will not accumulate, and leukocyte fragments will not be produced; however, clinical studies will be required to provide clinical evidence of benefit.

Question 6
The approach suggested by the BEST Working Party of the ISBT is a practical and logical way to validate a new filter and to provide ongoing assurance of process control [5]. They suggest that process validation be performed with a minimum of 20 consecutive products (60 if nonparametric measurements are used), to document successful implementation of a new product.
Inverted centrifugation at the time of transfusion is used as an alternative to filtration to prepare leukocyte-reduced red cell products for patients who have FNHTR. The threshold of leukocyte reduction that we are trying to achieve with this product is <5 × 10^8. Routine quality control on this product is problematic as counting procedures (automated and manual), for residual leukocytes in stored red cell products tend to be unreliable. We do perform automated leukocyte counts on the red cell products before and after leukocyte reduction. If the count on the leukocyte-reduced product is at the minimum counting threshold of the automated instrument (0.1 × 10^7/l), we assume that the process was effective in achieving our goal. However, clinical outcome (reaction/no reaction) is the most important indicator of process control for the preparation of this product.

A manual counting technique with a Neubauer counting chamber is used when leukocyte-reduced blood products are prepared by filtration [6]. This assay can detect to a threshold of 0.1 leukocytes/µl.

**References**


**Maurice Massé
Christian Naegelen**

**Question 1**
Leukocyte removal is the primary objective to be attained in order to enhance transfusion safety and patient comfort. Leukocyte depletion has been performed routinely for over 10 years on all standard and apheresis platelet concentrates. To date, in our regional hospital, over 80% of RBC concentrates are filtered prior to transfusion.

**Question 2**
Different filters are used for RBC concentrates: RZ200 B1H Sepacell (Asahi) and BPF4 BBSF (Pall) and two other types foruffy coat platelet concentrates and most apheresis platelet concentrates: PLS 5CB 2H Sepacell (Asahi) and LR F6 SC 2F (Pall). With these devices, in use for over 2 years now, we have achieved the objective of less than 1 × 10^7 residual leukocytes per blood component unit.

**Question 3**
Bedside filtration has never been performed in France. Numerous parameters may influence the outcome of leukocyte depletion [1]: filter capacity, flow rate, temperature, pH, or blood age. We think that the sole factor guaranteeing standardization of the filtration process, and hence blood component quality, is the laboratory control of this process. In several countries, the evolution is towards early filtration using a sterile connection device and filtration using in-line filters, to the prejudice of bedside filtration.

**Question 4**
Quality assurance pertaining to leukocyte depletion rests on good manufacturing practices for blood components. Each filter batch is inspected prior to its routine use, and several parameters are recorded on inspection sheets: temperature, flow rate, result of sterile connection inspection, filter performance. Qualified technicians are adequately trained. Internal audits are carried out to check blood component conformity with standards. These controls concern 0.6–3.5% of the total production, depending on the type of component. Furthermore, a national register of filtration quality controls has recently been created by the PSL working party: the evolution of the average quality of filtered RBC concentrates can thus be assessed in France.

**Question 5**
We believe that filtration must be implemented early if it is to be efficient [2]. European recommendations [3] advocate performing leukocyte depletion within 48 h postcollection. Depending on the time of collection, whole blood is stored at 20 °C for a few hours (5–8 h) or overnight (12–24 h). Our choice is based exclusively on logistic constraints.

**Question 6**
Any apheresis system, any new or modified filtration device must be validated prior to its routine use. The manufacturer’s claims are verified as part of multicenter studies, following strict process and control validation protocols. Five whole blood filtration devices have recently been assessed by 19 investigators belonging to the PSL group. This procedure accelerates national consensus about device performances and blood component characteristics. Recent publications [4], defining confidence and tolerance probabilities, have demonstrated the interest of validation. The parameters to be checked in the validation protocol must include process stability, capability and reproducibility, as well as blood component functionality and safety.

**Question 7**
Leukocytes are directly and efficiently removed from apheresis platelet concentrates using apheresis equipment (LRS Cobe Spectra or the new Fresenius AS104 protocol). Theuffy coat is currently removed from all RBC concentrates by centrifugation and automated decantation in bottom and top bags. In France, generalized filtration of RBC concentrates and whole blood, using in-line devices, planned for late 1997, will result in a single type of purified RBCs.
At our institution the blood collection is instituted into quadruple bags containing CPD as an anti-coagulant and saline-adrenaline-glucose-manitol as a red cell (RBC) additive/preservative solution. The target volume of collection is 450 mL ± 10%. Units of whole blood are centrifuged within 6 h of collection at 5,300 g for 6 min and fractionated by Compomat (NPBI, Emmmer-Compascuum, The Netherlands) into RBC, buoyy coat (BC) and plasma. We prepare platelet concentrates (PC) as follows: 6 BC are pooled with 350 mL of a crystalloid solution (F-Sol Platelet Additive Solution, Baxter, Deerfield, Ill.) and centrifuged at 570 g for 6 min. The supernatant PC is collected in a PVC bag if used within 6 h or in a polylefin bag (PL 2410, Baxter) if stored for a maximum of 5 days after donation.

Question 1
RBC and PC are not routinely leukocyte-reduced (LR). During 1996 leukocyte reduction was applied to 9% of RBC and 13% of PC. Specific indications for the use of LR RBC and PC are: (1) prevention of nonhemolytic, febrile transfusion reactions, (2) prevention of cytomegalovirus (CMV) infection in CMV-negative recipients belonging to the following categories: organ transplant if the donor is CMV-negative, intrauterine transfusion, perinatal/neonatal transfusion, other immunodeficiencies, and transfusion of pregnant women, and (3) prevention of HLA alloimmunization in kidney transplant candidates and transplantated patients, severe aplastic anemia, and thalasse mia.

Question 2
LR-RBC are prepared by filtration through RCVXLKLE (patient’s bedside filtration) or BPF4 (laboratory filtration; Pall, Portwashington, N.Y.). LR-PC are prepared by filtration through PL100E (Pall), both at the bedside and in the laboratory.

Question 3
During 1996, 78 and 27% of LR-RBC and LR-PC were filtered at the bedside, respectively. RBC and PC used in bedside filtration (as in laboratory filtration) are less than 7 and 3 days old, respectively. Most LR-PC are filtered in the laboratory because we find it very convenient to filter the PC in line at the time of production, after the centrifugation of the BC pool.

Question 4
Quality assurance of laboratory filtration follows the procedure described by Dumont et al. [1], which is based on process control rather than on postfiltration leukocyte count only. Briefly, each type of filter is first tested (n = 20–60) for conformance of leukocyte reduction obtained in our laboratory with those claimed by the manufacturer (process validation). Ongoing process control is then carried out with traditional quality control charts for mean and SD. Finally, the data generated during process validation and monitoring are used to determine the capability of the process to conform with leukoreduction standards. This is expressed as a claim that no more than a defined percentage of filtered components will contain more than a certain number of white cells. The claim is made with a specified level of confidence (usually 95 or 99%).

Because reliable postfiltration samples of bedside filtration are not available [2], we base quality assurance of bedside filtration on the qualification and training of nurses of the hematoloy department where virtually all bedside procedures are performed [2]. Furthermore, before starting their regular use in the clinic, RBC filters to be used at the bedside are tested in the laboratory for validation purposes after incubating the RBC unit at 27°C for 30 min [2], because bedside filtration can entail prolonged filtration times. This can cause an increase of the RBC temperature, which, in turn, is generally associated with a poorer performance of the filter.

Question 5
Under routine circumstances, RBC and PC are available for transfusion on the day after blood donation. Because white cells tend to disintegrate during storage and this can cause formation of debris and release of cytokines that can be variably trapped by current filters, we follow the prudent strategy of choosing for filtration 1- to 7-day-old RBC and 1- to 2-day-old PC.

Question 6
The filter should be tested with a number of units sufficient to reach statistically significant conclusions and with a method for counting residual white cells offering ade-
adequate sensitivity. With regard to the former, the number varies between 20 and 60 [1]. With regard to the latter, we believe that the Nageotte methods described by Prate et al. [3] and by Szuflad and Dzik [4] offer the best compromise of sensitivity and feasibility.

**Question 7**

No.

**Question 8**

Postfiltration leukocyte counts in LR-RBC are performed with a Nageotte chamber, following the 3% paraformaldehyde method described by Prate et al. [3] and validated by the Biomedical Excellence for Safer Transfusion (BEST) Working Party of the International Society of Blood Transfusion (ISBT) [5]. The lower limit of detection of this method is 50 white cells/ml, with a coefficient of variation of 21%. Residual leukocytes in LR-PC are counted with a Nageotte chamber as described by Szuflad and Dzik [4]. A preliminary evaluation of this method showed a lower limit of detection of 10 white cells/ml, with a coefficient of variation of 27.4% [4].

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**References**


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**Question 1**

In Finland we have used leukocyte-depleted red cells since 1979 and leukocyte-depleted platelets since 1985. The national quality requirement of <1 × 10^7 WBC/unit in at least 90% of units tested was adopted and met since 1985. The leukocyte-depleted components have been used to avoid HLA immunization and/or cytomegalovirus transmission and given only for the following groups of patients: (1) patients with leukemia and other hematological disorders, (2) kidney and other organ transplant patients, (3) small premature infants, (4) pregnant women and (5) when HLA-compatible components are used.

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**Question 2**

At this moment PPF4 (Pall) and LRT10 (Pall) filters are used for filtration of red cells and platelets, respectively. Whole blood filters have been under trial. Three types of whole blood filters have been validated and one of them will soon be taken into routine use.

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**Question 3**

All platelets have been filtered in the blood center. In 1990–1995 part of the red cells were filtered at the bedside. The practice decreased rapidly during 1996 and has now been stopped.

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**Question 4**

Filtration is centralized in five regional centers, all following the same SOP. QC samples are all assayed in the same laboratory, frequency of controls as advised by the Council or Europe [1]. Process parameters followed are weight, age and temperature of the buffy coat (BC)-free red cells used as starting material as well as duration of the filtration.

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**Question 5**

BC-free red cells are prepared from units of whole blood kept at room temperature after various storage periods (maximum 18 h). They are then stored overnight (minimum 16 h) at +4°C before filtration. Cold storage guarantees more effective removal of leukocytes. If the filtration is performed without cold storage the finished product will not meet the quality requirements.

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**Question 6**

We always do primary validation with 20 filters under strictly standardized conditions. If the results are then satisfactory, a series of trials (20 filters in each trial) are planned to test the robustness of the method and to validate some critical parameters which may vary in practice. On the basis of the results the final SOP is defined and validated with 20 filters, put into use and each of the first 50 units is checked.

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**Question 7**

No other methods are routinely used for leukocyte depletion.

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**Question 8**

Microscopic counting by the Nageotte chamber method a.m. Masse is used for routine QC. Flow cytometry is used for research purposes to type the remaining leukocytes with the aid of monoclonal antibodies.

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**Reference**


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Question 1
All red blood cell concentrates (RCC) prepared by the Red Cross Blood Bank Ulm are buffy-coat-depleted, which reduces the residual number of leukocytes in the RCC to approximately 10% and to an average number of 3 × 10^6 leukocytes. The random platelet concentrates (PC) prepared by our buffy coat method contain about 2 × 10^7 leukocytes per single random PC.

In 1996, about 160,000 RCC were delivered to our regional hospitals, approximately 9,000 (6%) were prepared as leukocyte-depleted RCC. In the same year, about 25,000 random PC from single blood donors were prepared. In addition 2,500 pools of 4–6 random PC were filtered and transfused as leukocyte-reduced PC (<1 × 10^5). The proportion of leukocyte-depleted components was quite different for the University Hospital Ulm with a large and active department of clinical hematology and stem cell transplantation [17,869 RCC and 4,147 leukocyte-depleted RCC (23%); 13,250 random PC and 9,300 filtered PC (70%)].

Nearly 1,200 PC were prepared by plateletpheresis in our blood center last year. The number of leukocytes in these preparations was <5 × 10^6 without filtration using the Cobe Spectra machine.

The cost of filters and the equivocal data regarding the benefit of leukocyte depletion in the daily routine of surgical patients are determining factors, not to use filtered RCC routinely.

Leukocyte-reduced blood components are indicated for patients with acute leukemia and lymphoma, who are treated with a curative aim, with aplastic anemia, with paroxysmal nocturnal hemoglobinuria, with osteomyelofibrosis, with myelodysplastic syndrome, before and during stem cell transplantation, before, during and after kidney transplantation, with hereditary anemia, organ transplant recipients, pregnant women and fetuses (intravenous transfusion) and infants below 12 months of age, with severe nonhemolytic, febrile transfusion reactions after transfusion with buffy-coat-depleted RCC, and who are CMV antibody-negative and severely immunosuppressed.

Our current transfusion strategy with regard to patients with acute leukemia or lymphoma irrespective of the possibility of bone marrow or peripheral stem cell transplantation is to transfuse them with leukocyte-depleted RCC or PC [1, 2]. When these patients develop antibodies to HLA leading to refractoriness to platelet transfusion, HLA-compatible PC prepared by plateletpheresis are transfused.

Question 2
RCC are filtered by BPF4-BBSD, Pall, Medsep and pooled random PC by LRP6-SE, Pall, Medsep and PC prepared by pooled buffy coats by AUTOSTOP BC, Pall, Medsep.

Question 3
Filtration of all our blood products is performed in the blood bank. Because the result of leukocyte filtration is influenced by so many factors, the filtration procedure used should be strictly standardized. The filtration at the bedside carries the risk of undesired results as untrained personnel may vary procedures of the filtration process. Thus an adequate quality control of the filtered blood component is not achievable.

Question 4
Quality checks of filtered RCC include volume, hematocrit, hemoglobin, loss of red cells, sterility, and at the end of the storage period, potassium and hemoglobin in the supernatant (plasma and SAGM). Quality indicators for the filtered PC are volume, residual leukocytes (counted in 1% of concentrates), platelet recovery, the swirling effect of the PC and the pH at the end of the storage period.

Question 5
RCC are filtered on the 2nd to 3rd day postcollection. The maximum prefiltration storage period allowed in our blood bank is 5 days. Leukocyte removal by red cell filtration is significantly influenced by the age of the RCC. In addition the RCC should always be filtered as soon as possible after collection to prevent the release of antigenic fragments and possibly virus or bacteria released from disintegrating leukocytes. However, soluble HLA antigens are much less immunogenic than the same antigens on the surface of intact leukocytes. Therefore some delay in filtration related to HLA immunization has no serious consequences.

Question 6
New filters should be validated according to the guide to the preparation, use and quality assurance of blood components of the Council of Europe regarding residual contaminating cells [2]. In addition the yield and the function of the cells after filtration and storage should be examined. Determination of cytokine levels of stored filtered blood products may also be helpful in the quality control of PC [3].

References

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Question 1
Our blood bank serves about 5,500 hospital beds with 90,000 donations annually. About 25% (n = 20,000) of the red cell concentrations (RCC) are issued leukocyte-depleted and 99% (n = 40,000) of the platelet concentrates (PC). The majority are used in two university hospitals and a cancer center. Main indications are (1) prevention of HLA alloimmunization (patients with hematological disease who will be dependent on platelet transfusions, patients awaiting and during a bone marrow or peripheral stem cell transplant or awaiting a renal transplant), (2) prevention of cytomegalovirus transmission (transplant patients, pregnant women, premature with a birth weight below 1,500 g, and (3) prevention of nonhemolytic febrile transfusion reactions.

Due to the increasing costs of health care, debates are ongoing on whether or not other indications concern patients with myelodysplastic syndromes, hemoglobinopathies, refractory leukemia and prevention of immunomodulation (surgery for colorectal carcinoma).

Question 2
Since 1975 we have used the Cellselect cellulose acetate filter from NPBI for red cell filtration. Having processed leukocyte-depleted single donor PC from buffy coat (BC) for 10 years, in 1995 we switched to preparing pools of 5 BC and plasma from one unit. The platelet-rich plasma (PRP) from these pools is leukocyte-depleted during transfer to the platelet storage bag with an Autostop (Pall) filter.

Questions 3 and 4
All filtrations are performed in the blood bank applying validated standard procedures by trained personnel. Quality control is at random because of the excellent performance of the filters giving 99% confidence that the filter removed leukocytes to fewer than $5 \times 10^5$ and in 99% fewer than $1 \times 10^3$ (n > 1,000, none failing). For quality control, 1% of the filtered components are checked for the residual number of leukocytes.

Question 5
Whole blood is stored overnight under butane-1,4-diol cooling plates at 20–24°C. After approximately 16 h high spin centrifugation is applied and the blood is separated into plasma, BC and RCC in SAGM. RCC are filtered within 24 h of collection. Occasionally, when RCC with special blood types are needed older units are filtered. BC are pooled 2–4 h after separation. PC are always filtered within 24 h of collection. The choice for the overnight storage of whole blood has been made for logistical reasons, better platelet yields and better centrifugation conditions.

Question 6
A standard operating procedure should be written according to the manufacturer’s instruction and including the handling of the filter, the composition of the component, the age (time from blood collection to separation and to filtration, respectively), the temperature of the blood component, flow rate, height, labelling and quality control checks. Following training of the personnel, validation of a selected number of filtrations should be performed for which criteria should be set regarding maximum number of residual leukocytes ($5 \times 10^3$) and the accepted percentage of failures. The filter performance will be judged according to Dumont et al. [1]. If accepted, the filtered components should be validated for storage after filtration in the storage bags which are to be used. Especially for platelet measurements for activation and cytokine formation are necessary. Platelet activation before or during filtration can lead to unexpected platelet loss. For red cells hemolysis induced by filtration should be carefully checked as well as red cells captured in the filter and/or tubing.

When the filter is in continued use, the quality control should first be intensive in order to increase the number of validations. When leukocytes have been counted in 200 filtrations without failure, quality checks can be reduced to 1%.

Question 7
As stated before all RCC are BC-depleted having a residual leukocyte count below $1.2 \times 10^3$. For PC, a blood separator (Compomat, NPBI) can be used to express the PRP very slowly and to stop when red cells are detected. With this method 85–90% of the PC contain fewer than $5 \times 10^5$ leukocytes. Although the costs of the filters are saved, 100% of the pools have to be counted which is laborious and therefore also costly.

Question 8
For counts equal to or below $5 \times 10^5$ in a 300-ml volume, the Nageotte hemocytometer is used for both red cells and platelets in a 1:5 dilution in Leucoplate. The precision of this assay is 0.1 cell/µl.

Reference

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Question 1
A recent survey of hospitals served by the London and South East Zone of the National Blood Service (860,000 donations/year) indicates that approximately 10% of red cells and 18% of platelets are leukocyte-depleted. The main indications are intra-uterine and neonatal transfusions to avoid possible short-term and long-term immunosuppressive effects, prevention of HLA alloimmunisation in patients with severe aplastic anaemia who may in the future have an allograft, patients awaiting renal transplantation (unless part of an immunosuppressant protocol), and prevention of cytomegalovirus (CMV) transmission if CMV-seronegative components are unavailable.

For primary prevention of febrile reactions, we recommend the use of buffy coat-depleted red cells or platelet pools derived from buffy coats as the most cost-effective first measure. Leukocyte depletion is reserved for those patients with ‘break through’ reactions despite the use of these products.
Leukocyte depletion is not routinely recommended in our practice for patients with hematological or other malignancy, or for bone marrow/stem cell transplant recipients, except as discussed above.

Question 2
Within our 3 production sites, Pall BPF4 and Autostop are used for ‘in-process’ leukocyte depletion of red cells and pooled platelets, respectively. There is increasing interest in whole blood filtration, and both Pall and Asahi filters have been evaluated in this regard.

Our policy is to recommend prestorage leukocyte-depletion where the objective is to prevent HLA alloimmunisation or CMV transmission. Similarly, bedside filtration has been shown to be ineffective in preventing febrile reactions to platelets. However, bedside filtration does have a role in the prevention of febrile reactions to red cells where buffy coat-depleted red cells may be inappropriate, e.g. hemoglobinopathy patients. For other patients with troublesome reactions to buffy coat-depleted red cells, prestorage filtration is more likely to be effective.

Question 3
Our recent survey of 47 hospitals indicates that 45% of hospitals obtain all leukocyte components from blood centers, 11% perform all leukocyte depletion at the bedside, and 40% use both approaches, depending on the clinical indication.

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Question 4
The 3rd edition of the UK Guidelines for the Transfusion Services requires that 100% of leukocyte-depleted units have a leukocyte count of <5 × 10^6 leukocytes/unit. However, counting of every unit is not required for well-validated filtration systems. Once an initial validation has demonstrated consistency, only 1% of filtered units need to be sampled and counted as described below. Statistical process control is used to ensure that the system is in control.

Question 5
For blood centre filtration of processed red cells, we aim to filter no later than 48–72 h after collection; whole blood filtration will be performed within 24 h of collection. Platelets produced from whole blood are generally filtered during processing, i.e. within 24 h of donation. We have not defined a minimum ‘hold’ time between collection and filtration, but this is rarely carried out less than 2 h after collection.

Early filtration is most effective in preventing a rise in cytokine levels seen during storage of platelets and provides maximum opportunity to remove intact cells prior to cellular disintegration.

Question 6
Any system must be able to provide components which consistently fulfill the appropriate product specification for volume and haematocrit/platelet count/pH as well as being leukocyte-depleted to 5 × 10^6 leukocytes/unit. This will involve 100% testing of a predefined number of units. Ideally, more than 1 batch of filters should be evaluated. The optimal conditions need to be established for each filter, e.g. interval between collection and processing, flow rate and for whole blood, the prefiltration storage temperature.

Whole blood filtration also requires consideration of the plasma quality, and tests for markers of complement activation and thrombin generation are appropriate. Additional markers are under evaluation in many laboratories, e.g. cytokine generation or removal by filters, markers of platelet activation, and leukocyte subset analysis. These have not yet found a place in routine evaluation of filters and other apheresis techniques, although this is a rapidly evolving field.

Leukocyte depletion of platelets by COBE LRS apheresis technology was implemented in the zone during spring/summer 1997. This component is leukocyte-depleted during collection of the platelets. On implementation, 100% testing of components collected on each machine was performed till consistency is established. A national evaluation of bacterial safety will also be performed.

The zone currently uses both Nageotte chamber counting and flow cytometry using the Ortho Cytoron, which calculates an accurate leukocyte count from a precisely measured sample volume delivered via a Hamilton syringe. The Nageotte method has been reported to detect 2 WBC/μl with a CV of <23%, while the flow cytometry method can detect <10 WBC/μl.

Question 7
Leukocyte depletion of platelets by COBE LRS apheresis technology was implemented in the zone during spring/summer 1997. This component is leukocyte-depleted during collection of the platelets. On implementation, 100% testing of components collected on each machine was performed till consistency is established. A national evaluation of bacterial safety will also be performed.

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References


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Question 1
Leukocyte-depleted RBC (LD-RBC) and leukocyte-depleted single donor plateletphe

leukocyte depletion where the objective is to

LD-SDP are not used routinely because of cost. The cost of LD-RBC is 49% higher than standard RBC and LD-SDP is 6% higher. Currently, 9% of RBC and 6% of SDP are issued prestorage leukocyte-depleted. RDP make up only 20% of platelet doses; we do not currently offer leukocyte-depleted RDP.

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Orders for leukocyte-depleted products are most often from oncologists. The primary indication for LD-SDP is the avoidance of alloimmunization and for LD-RBC is prevention of recurrent febrile nonhemolytic transfusion reactions (FNHTR). Avoidance of alloimmunization is of primary concern to physicians caring for patients requiring intensive transfusion support [1]. The most recent estimates of the multi-institution clinical Trial to Reduce Alloimmunization to Platelets (TRAP) have confirmed the evidence that refractoriness to platelet transfusion decreases with the use of LD-SDP: from 13 to 4% [2].

Patients with hematologic malignancies such as leukemia, lymphomas, aplastic anemia or multiple myeloma, and all patients receiving bone marrow transplantation routinely receive LD products. Patients with solid tumors, at the nadir of their chemotherapy, are expected to require fewer transfusions and usually do not receive LD products. In addition, oncologists will order LD products in an effort to prevent cytomegalovirus (CMV) infection in patients receiving bone marrow transplantation since these patients are at special risk of developing devastating CMV disease [3, 4]. The decreased risk of CMV transmission is also an indication for the use of LD products for neonatologists. Interestingly, hospitals with a large population of AIDS patients have converted to 100% leukocyte-depleted products in response to the possibility of reactivation of recipient viral infections [5, 6].

Physicians also order LD-RBC for patients with recurrent FNHTRs. Current data also suggest that filtration of platelet products within 1–2 days of production decreases the concentration of plasma cytokines, reducing the incidence of recurrent FNHTRs following platelet product transfusion to levels equal to those of LD-RBC products [7, 8]. Many of our physicians are interested in the potential usefulness of prestorage filtration in preventing FNHTRs following platelet transfusion.

The use of LD products to reduce transfusion-mediated immunomodulation is more controversial; fewer physicians order LD products for this indication [9, 10].

**Question 2**

For RBC filtration we use the Baxter-Fenwal Sepacel™ Pre-Storage Leukocyte Reduction Set (product code No. 4710/4360). The residual (log) mean WBC count for 1996 was 4.55±0.60 per unit. For SDP filtration we previously used the Fenwal CS3000™ with Asahi PLSSA™ filters integral to the kits (product code No. 12710). In 1996 we converted to the COBE LRS™ (product code No. 12710). The (log) mean residual WBC count using leukofiltration was 5.14±0.61 per unit (1995) and for the COBE LRS was 4.55±0.41 per unit (1996).

Because of concerns over filtration relating to reduction in platelet size [11], potential for bradykinin activation [12] and difficulty achieving 85% platelet recovery, the IMBC no longer uses membrane filtration for SDP leukodepletion. This also has had the effect of reducing the fraction of units exceeding 1×10^6 WBC/unit from 10 to 1%.

**Question 3**

We estimate, using industry (1995) and IMBC sources, that in California 25% of total RBC and 73% of total SDP are transfused leukocyte-depleted. Approximately 64% of RBC leukocyte-reduction and 92% of SDP leukocyte reduction are achieved by bedside filtration.

**Question 4**

Because the distribution of residual WBC counts in leukocyte-depleted RBC and SDP units shows little variability, and the (log) mean of residual RBC is 2 logs below cutoff, we have 99% confidence that at least 95% of the units will meet the European standard of 1×10^6 WBC/unit as well as the FDA-mandated standard of 5×10^6 WBC/unit. To assess process control over time we sample 5 LD-RBC and 4 LD-SDP per month per site. We are currently evaluating the power of this sample size to detect a significant shift in process capability.

Our evaluation of the COBE LRS provides an example of how we assure the quality of our products [13]: (1) Process validation: the distribution of 20 log-transformed residual WBC counts was evaluated with a cumulative frequency plot: the units were log-normally distributed and 100% of the units contained 1×10^6 WBC’s. (2) Capability assessment and conformance with Standards [13]: the (log) mean of the 20 initial WBC counts was less than the ‘maximum acceptable’ (log) mean WBC count, assuring us that the distribution of residual WBC’s was in conformance with standards. The ‘maximum acceptable’ (log) mean WBC count is calculated as the (log) standard [k7×(log)SD] where the cutoff of 1×10^6 WBC/unit is used, and k7, a tolerance factor that varies with the desired degree of confidence and tolerance, is found in a tolerance table [13]. Periodic assessment of WBC mean and variance with increasing sample size continues to give us 99% confidence that 99.5% of the components meet the standard of ≤1×10^6 WBC/unit. (3) Ongoing process control: the stability of monthly residual WBC counts are monitored and inspected for nonrandom events and drift.

Quality assurance of units that are filtered at bedside is performed by the transfusion service and usually includes only initial validation of the product. Ongoing process control is sporadic. Quality control of bedside filtration is not routinely performed.

**Question 5**

We follow the manufacturer’s instructions for time recommended between RBC donation and filtration: greater than 12 h and lesser than 5 days. We typically filter at day 3 because marker testing has been completed and less hemolysis was observed than after filtration was 4.55±0.60 per unit. Because of concerns over filtration relating to reduction in platelet size [11], potential for bradykinin activation [12] and difficulty achieving 85% platelet recovery, the IMBC no longer uses membrane filtration for SDP leukodepletion. This also has had the effect of reducing the fraction of units exceeding 1×10^6 WBC/unit from 10 to 1%.

**Question 6**

A draft of a standard user validation approach is being developed for evaluation by the BEST subcommittee of the ISBT. This is expected to be ready by 1998.

**Question 7**

In order to maximize process control, only those methods of leukocyte reduction listed in response to question 2 are utilized routinely.
**Question 8**

Residual WBC in LD-RBC are quantified using PCR. The lower limit of detection (sensitivity limit) is 1 WBC/125 µl (8 cells/ml). The coefficient of variation at the lower limit is 50–60% [14]. The high CV most likely represents random sampling error: the observed %CV is similar to that predicted by Poisson analysis [15].

Residual WBC in LD-SDP are quantified using a Nageotte hemocytometer. The lower limit of detection for the technique is 0.1 WBC/µl (3 x 10^4 WBC in a 300-ml product) [16], the CV in the lower range is 21–50% [17]. The accuracy level is 1 WBC/µl (3 x 10^5 WBC in a 300-ml product) [16]. In the range of clinical interest (1 x 10^6–7 WBC/unit) the CV is 8.6% [17].

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**References**


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