BACKGROUND: Before-storage WBC reduction by filtration appears to be an effective way to prevent transfusion-associated complications. It also has superiority over WBC reduction at the time of transfusion (bedside), due to the many variables associated with such practice and the difficulty in performing adequate quality control. To determine the adaptability of collection systems containing in-line filters to the current blood collection strategy, the feasibility, efficiency, and quality of before-storage WBC reduction of whole blood (WB) units were evaluated, following their prolonged storage at ambient temperature prior to component preparation, by use of an integral in-line filter.

STUDY DESIGN AND METHODS: Blood was collected from random donors into quadruple blood pack units with an integral in-line filter and divided into three groups, according to storage conditions. WBC reduction was performed at room temperature, on WB units after storage at ambient temperature either for less than 8 or up to 18 hours on 1,4-butanediol cooling trays or for 18 hours in the cold.

RESULTS: All the filtration procedures met the AABB threshold of less than $5 \times 10^6$ residual WBCs per unit and the European requirements for WBC counts of less than $1 \times 10^6$ WBCs per unit. The average filtration time was less than 22 minutes in all units studied. Filtration time and blood flow rate were both significantly longer, and RBC loss was significantly higher in WB units that were filtered after prolonged storage in the cold.

CONCLUSIONS: Adequate before-storage WBC reduction can be achieved when performed on WB units, which were stored at ambient temperature for 18 hours, by use of an in-line filtration system. The procedure, performed under relatively simple logistics, results in good-quality, standard components, which require no further modifications when supplied to the transfusion services.

The removal of WBCs from cellular blood components is associated with reduction of several transfusion-associated adverse reactions. These include febrile, nonhemolytic transfusion reactions; HLA alloimmunization with subsequent refractoriness to platelet transfusions; transfusion-associated GVHD; immunosuppression; and transmission of certain infectious leukotrophic agents such as CMV and HTLV I/II. The transfusion of WBC-reduced blood components has become a common practice, at least for selected patient groups, in many countries. Moreover, some have decided to implement a policy of universal WBC reduction to all blood units and components.

WBC reduction at the time of transfusion (bedside), widely used in many centers, has been shown to be less efficient in preventing complications, such as febrile, nonhemolytic transfusion reactions that result from cytokines accumulating during blood component storage. In addition, the many variables associated with bedside WBC reduction and the difficulty in performing adequate quality control of the filtered components make filtration before storage, within 24 hours after collection, the preferred method.

Implementation of the filtration procedure, either at the level of the central blood services or at transfusion centers ("laboratory WBC reduction"), requires substantial changes in the logistics and deployment of the entire blood component preparation system, especially where large collection facilities are involved.

Magen David Adom (MDA) National Blood Services are responsible for the collection of 260,000 blood units yearly, from volunteer donors, nationwide. About 88 per-

ABBREVIATIONS: MDA = Magen David Adom; RT = room temperature; WB = whole blood.

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percent of the units are collected in mobile blood drives, while the additional 12 percent are drawn in fixed-site donor rooms at MDA first-aid stations. All units are transferred daily to two major laboratories, for further processing into blood components. According to the transfusion services demands, 96 percent of the whole blood (WB) units will be separated into RBCs and FFP, while only 50 percent will be further processed into platelet units.

To enable standardization of all blood units, the collected WB units are immediately placed on special 1,4-butandiol cooling trays (Compocool, NPBI, Emmer-Compascuum, the Netherlands) and kept at room temperature (RT) (20–24°C) until processing. About 40 percent of all the units are separated into components within 8 hours after collection; the rest will be processed after being kept on the cooling trays for up to 18 hours after donation.

The aim of this study was to evaluate the feasibility, efficiency, and quality of before-storage WBC reduction of WB units, following their prolonged storage at ambient temperature, by use of collection systems with in-line filters.

The outcome of this study may have an important impact on the logistics needed to provide before-storage WBC-reduced blood units of high quality to transfusion services.

MATERIALS AND METHODS

Blood collection and component preparation
WB units were collected from 60 random donors with manual mixing, into quadruple blood packs units (4R3316, Baxter Healthcare Corp., Deerfield, IL), with an in-line filter for WB (RZ2000, Ashai Medical Co., Tokyo, Japan). Each system included a collection bag containing 63 mL of CPD and a satellite bag containing 100 mL of RBC preservation solution (Adsol, Baxter Healthcare Corp.).

Following donation, the collected bags were divided into three groups of 20 units each: Groups A and B were placed under cooling plates (Compocool, NPBI) containing cooled 1,4-butandiol, to ensure rapid cooling to 22 ± 2°C, and kept at RT for 8 and 18 hours, respectively. Units in Group C were processed into components after 18 hours of storage at temperature of 4 ± 2°C.

Filtration was performed on all units at RT, according to the manufacturer’s instructions. After in-line filtration, the WB units were centrifuged at 4000 × g for 5 minutes at 22 ± 2°C in a centrifuge (Sorvall RC3-BP, Kendro Laboratory Products, Newtown, CT) and were further separated into plasma and RBCs by use of semiautomated blood component extractors (Compomat G3 system, NPBI). The plasma was then transferred into an integrated satellite bag, rapidly frozen to −30°C, and stored as FFP at −30°C. The RBCs were resuspended with Adsol solution and stored at 4 ± 2°C.

WB and component evaluation
Volumes of WB units and blood components were determined by dividing the net weight by standard specific gravity values.

Hct values and WBC and RBC counts were evaluated on samples taken from the WB and the processed RBC units by use of a counter (Cell Dyn 1600, Abbott, Abbott Park, IL).

Residual WBC counts in the filtered RBC units were performed by use of the Nageotte hemocytometer as follows: 50 µL of filtered RBC samples was diluted 1 in 10 by adding 400 µL crystal violet solution and 50 µL of lysing agent (Zap-O-Globin, Coulter Corp., Hialeah, FL). After staining, samples were transferred to a Nageotte hemocytometer, allowed to settle for 30 minutes in a humidified atmosphere, and counted by use of a light microscope.

Statistical analysis
Results are presented as mean ± SD. The results were compared by use of the paired t-test. A p value less than 0.05 was considered significant.

RESULTS

Before and after filtration measures
The average volumes of all 60 WB units (including 63 mL of CPD) and their cellular contents (RBCs and WBCs) before and after filtration are shown in Table 1. There was no statistical difference between the measures in the three groups tested, prior to filtration. Similarly, the volumes of the WB after filtration and their derived RBC units were not significantly different, among the three groups.

Filter performance
Assessment of WBC reduction efficiency by use of the in-line filter system is depicted in Table 2. The extent of

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before filtration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WB volume (mL)</td>
<td>473 ± 16</td>
<td>470 ± 15</td>
<td>468 ± 18</td>
</tr>
<tr>
<td>RBCs (×10^12)</td>
<td>2.10 ± 0.25</td>
<td>2.09 ± 0.28</td>
<td>2.30 ± 0.36</td>
</tr>
<tr>
<td>WBCs (×10^9)</td>
<td>2.52 ± 0.71</td>
<td>2.94 ± 0.67</td>
<td>2.66 ± 0.65</td>
</tr>
<tr>
<td>After filtration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WB volume (mL)</td>
<td>442 ± 18</td>
<td>436 ± 16</td>
<td>436 ± 20</td>
</tr>
<tr>
<td>Adsol RBC Hct (%)</td>
<td>54.6 ± 3.6</td>
<td>55.2 ± 2.6</td>
<td>55.7 ± 2.1</td>
</tr>
<tr>
<td>RBCs (×10^12)</td>
<td>1.85 ± 0.23</td>
<td>1.81 ± 0.22</td>
<td>1.85 ± 0.30</td>
</tr>
</tbody>
</table>

* Results presented as the mean ± SD.† WB stored under cooling trays at RT for up to 8 hours.‡ WB stored under cooling trays at RT for 18 hours.§ WB stored at 4 ± 2°C for 18 hours.
**TABLE 2. In-line filter system performance***

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBCs (x10⁶)</td>
<td>0.69 ± 0.38</td>
<td>1.54 ± 0.87††</td>
<td>0.81 ± 0.35</td>
</tr>
<tr>
<td>Range (x10⁶)</td>
<td>0.21-1.5</td>
<td>0.45-3.1</td>
<td>0.29-1.5</td>
</tr>
<tr>
<td>Number of units ≤5 x 10⁶</td>
<td>20 (100%)</td>
<td>20 (100%)</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>Number of units ≤1 x 10⁶</td>
<td>20 (100%)</td>
<td>20 (100%)</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>Number of units ≤0.5 x 10⁶</td>
<td>20 (100%)</td>
<td>20 (100%)</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>Log WBCs reduction</td>
<td>4.36</td>
<td>4.19</td>
<td>4.33</td>
</tr>
<tr>
<td>Filtration time (min:sec)</td>
<td>12:34</td>
<td>12:32</td>
<td>21:59‡</td>
</tr>
<tr>
<td>Flow rate (mL/min)</td>
<td>44</td>
<td>41</td>
<td>26‡</td>
</tr>
<tr>
<td>RBCs recovery (%)</td>
<td>88</td>
<td>87</td>
<td>80‡</td>
</tr>
</tbody>
</table>

* Mean ± SD.
† Group B versus Group A and Group C; p < 0.05.
‡ Group C versus Group A and Group B; p < 0.05.

**DISCUSSION**

In view of the trend for universal WBC reduction, collection systems are designed to provide blood services with easy, cost-effective, and less labor-intensive methods to prepare WBC-reduced components. Such collection systems, containing an integral filter, would be useful if they enable the implementation of universal filtration procedures, without the need to change the entire component preparation logistics.

So far most of the commercially available filters have been designed to perform WBC reduction after either a short storage at RT (<8 hr) of the WB units or a more prolonged storage of the RBCs in the cold. Acceptable filtration results were reported for WB units either after they were kept in the cold or following prolonged storage at ambient temperature. However, WBC reduction in the latter study was performed on RBCs whose buffy coat layers were previously removed (by use of a “top and bottom” separation method) and not on unmodified WB, prior to its separation into components. In addition, laboratory filters rather than integral filters were used.

In Israel, close to 96 percent of all units collected by MDA National Blood Services are placed on cooling trays immediately after donation and stored at ambient temperature, similar to the method used in some European countries. This practice enables us to achieve standardization of blood units collected nationwide, at different environmental temperatures (as well as other conditions). Storage time before processing may vary from 8 to 24 hours after collection, providing high-quality blood components, without the need to implement high-cost mobilization systems and a laboratory night shift if processing the blood within 8 hours is required.

Anticipating the growing demand for the supply of before-storage WBC-reduced blood components, and in view of the logistics required to meet such requests, we studied the feasibility, efficiency, and quality of WB filtration, by use of a blood collection system with an in-line filter.

To the best of our knowledge, this is the first validation of such a system in previously unmodified WB units stored under these conditions.

Our results show that the integral system studied is capable of meeting both the AABB standards of less than 5 x 10⁶ WBCs per unit and the Council of Europe guidelines of less than 1 x 10⁶ residual WBCs per unit, in all units tested.

The studied system removes all the platelets from the filtered WB units, similar to other currently available WB and RBC filters. As a result platelet concentrates cannot be prepared, by use of this in-line filtration system. Whenever platelets units are needed, they should be made from blood units that were separated prior to filtration.

All the in-line filtration procedures were performed within reasonable times and rates. However, WB units which were kept longer in the cold prior to filtration showed a significant increase in the filtration rate and a
decrease in RBCs recovery, when compared to units stored at RT for similar periods of time. These differences can probably be attributed to different viscosity or to formation of microaggregates during cold storage.

According to our experience, adequate WBC reduction prior to storage can be achieved when performed on WB units, which were stored at ambient temperature for 18 hours, by use of an in-line filtration system. The procedure is routinely used in Israel, as it results in good-quality, standard components, which require no further modifications when supplied to the transfusion services nationwide.

REFERENCES