Leukocyte Depletion and Recovery with Pall Sterile WBC Syringe Filter

Emily Nicholson1, Satbeer Singh2, Alun Gordon2, Laura Puddy2, Hiromi Gonzalez Fuentes1, Maria Martinez Lobete1
1PALL Corporation; 2Clinical Flow Cytometry, Department of Blood Sciences, Queen Alexandria Hospital, Per

1. Summary

Pall’s sterile Acrodisc® white blood cell (WBC) syringe filter (Part numbers AP-4951 and AP-4952) has been re-designed and optimized to better fit the requirements of WBC separation workflows. This syringe filter is now supplied sterile (gamma irradiated) to help prevent unwanted contamination, and includes a female leu lock (FLL) outlet that replaces the male slip leu outlet (MSL) connection, thus better facilitating the backwash step to recover the WBC’s. Additionally, the Acrodisc WBC syringe filter now includes color coding hardware (white outlet) so that orientation is obvious during use.

Pall’s sterile Acrodisc WBC syringe filter is a proven filtration device designed to capture and recover leukocytes for further analysis of WBC’s, alternatively, removal of WBCs to produce leukodepleted blood samples. This is an established tool for processing up to 12 mL of whole blood samples in research laboratories equipping scientists with a simple and efficient method to isolate or remove white blood cells. Moreover, the Acrodisc WBC syringe filter enables rapid and safe processing of clinical blood samples for research applications which require white blood cell recovery, leukocyte depleted blood, and recovery of viable WBC for in situ lysis for nucleic acid purification.

The cell separation performance of the sterile Acrodisc WBC syringe filter is based on the unique separation capability of Pall’s proprietary leukosorb media, a fibrous matrix designed for use in procedures requiring isolation or removal of leukocytes from whole blood samples.

In this application note an evaluation of the performance of the sterile Acrodisc WBC syringe filter was conducted by means of RBC recovery (leukocyte depletion) in the filtrate, as well as WBC recovery and WBC viability before and after filtration of human whole blood samples. In the Leukocyte depletion experiment, the flow cytometry data showed over 99% removal of WBC from the samples in all cases studied. Additionally, Acrodisc WBC syringe filter is able to recover approximately 60% of WBC after the back-wash step, and the viability of the recovered leukocytes is approximately 80%.

Results show that the filter maintained claims under all sample testing conditions i.e., non-gamma and gamma irradiated Acrodisc WBC syringe filters at time zero and at time two, which is the equivalent to two-years old of accelerated ageing. This accelerated aging test was performed on 20 gamma irradiated and 20 non-gamma irradiated Acrodisc WBC syringe filter units, to potential identify any changes to the Acrodisc post gamma irradiation and ageing.

In all test cases values from the non-gamma irradiated samples were set as a control to determine the potential impact of the irradiation on the performance of the filter units.
2. Objective

The purpose of this study was to validate the performance of the new sterile Acrodisc WBC syringe filter by measuring the leukodepleted filtrate, as well as the WBC recovery and viability of the backwash elution sample. By studying the cell viability, the overall condition of the cells recovered and lost can be analysed and therefore, identify potential cell damage due to the filtration process. The shelf life claim was validated by accelerated shelf life testing to the equivalence of two years, by storing the syringe filters in a 50 °C oven.

The integrity of the syringe filters before and after gamma irradiation was also measured. This test determines whether the integrity of the media and housing have been affected due to gamma irradiation or throughout the product's shelf life.

3. Materials and Methods

a. Cell Recovery and Viability Test

For this test, 20 gamma irradiated and 20 non-gamma irradiated Acrodisc WBC syringe filter units were tested to validate the WBC viability and recovery, as well as to track any changes that the gamma irradiation could cause to the filter device.

The samples analysed for this scientific application note were whole human blood samples, collected and preserved using EDTA coated vacutainers. All samples were stored and transported at room temperature; they were vortexed after collection and before the filtration step. All samples were tested within 36 hours after collection to prevent WBC viability to decrease below 80%. WBC concentration was determined by flow cytometry before and after filtration of the whole blood samples. This entire process required i) whole blood analysis, ii) whole blood filtration, iii) filter back wash, and iv) flow cytometry sample preparation. The test was performed by the Clinical Flow Cytometry Group from the Department of Blood Sciences at Queen Alexandria Hospital, Portsmouth, UK.

i. Whole Blood Analysis

K2EDTA treated whole human blood in samples (Cambridge Bioscience, UK) were analysed by flow cytometry to determine the initial WBC concentration. For this analysis, 100 µL of the EDTA whole human blood was transferred into a CD45 coated tube (DuraClone IM Cell Count Kit, Beckmann Coulter, US). Each tube was vortexed for 30 seconds and incubated for 15 minutes at room temperature. After the incubation period, 2 mL of red blood cell lysis buffer (VersaLyse® Beckman Coulter, US) was added to each sample, vortexed for 10 seconds and incubated 15 minutes at room temperature. Finally, WBC concentration was determined by flow cytometry. These obtained values represent the initial WBC count.

ii. Whole Blood Filtration: Flow Cytometry Sample Preparation

To start the filtration process, the sterile Acrodisc WBC syringe filter was removed from the blister packaging and the upstream side (transparent side with green writing) attached to a 10 mL syringe, from which the syringe plunger has been previously removed (See Figure 1.1 and 1.2). Once attached to the syringe, the Acrodisc WBC syringe filter was placed on top of a sterile 50 mL falcon test tube (VWR, US) and secured with tape (See Figure 1.3). Prior to filtration, the whole blood sample was mixed by vortexing for 10 to 15 seconds. Then 5 mL was transferred to the empty syringe barrel to proceed with filtration. The blood filtered through the Acrodisc WBC syringe filter by gravity and the filtration time was recorded. Once the 5 mL of whole blood was filtered, 10 mL of isotonic, flow cytometry compatible Phosphate Buffer Solution (PBS), was added to the syringe barrel and filtered by gravity to wash the membrane of the Acrodisc WBC syringe filter unit. The filtered PBS was collected into the previous falcon test tubes and the filtrate was labelled as “Leukocyte Depleted Sample” (See Figure 1.5). This sample was analyzed by flow cytometry to determine the WBC loss and viability.
Figure 1
Filtration procedure for WBC separation using the Acrodisc WBC Syringe Filter

1. Remove the Acrodisc WBC syringe filter from its blister packaging.

2. Remove the syringe plunger and attached the 10 mL syringe to the filter inlet.

3. Place on top of an open 50 mL collection tube and secure with tape.

4. Pour the whole blood sample into the syringe barrel and allow the blood to filter under gravity.

5. When the blood has filtered through, add 10 mL of PBS solution and filter by gravity.

6. The filtrate is the leukocyte depleted sample.

7. The syringe filter is flipped over and the outlet side is connected to a new 10 mL syringe, filled with PBS.

8. The filter with the syringe is placed on a new collecting tube, and the PBS is pushed through the filter.

9. Multiple backwash steps can be carried out. (3-5 repeats recommended to maximize WBC recovery).
iii. WBC Recovery
Leukocytes captured on the filter media were recovered into an elution solution using the back flush procedure. A back-flush is a reverse flow setup to elute the WBCs. For this, the syringe barrel was unscrewed and the Acrodisc WBC syringe filter was flipped over (reversed), as shown in Figure 1.6. Then a syringe was filled with 10 mL of PBS, connected to the white outlet end, and the plunger was pressed down to wash the WBC into a falcon test tube (see Figure 1.7). As depicted in Figure 1.8, backflush steps using in total 50 mL of PBS (between 3 and 5 syringe refills) was carried out to maximize the leukocyte recovery. The filtrate collected was pooled, vortexed and then labelled as “WBC Recovery Sample”.

Finally, the “WBC Recovery Sample” and the “Filtrate Sample” were ready for further downstream analysis. In this case samples were prepared for flow cytometry analysis as described in above section ‘whole blood analysis’. This analysis was to identify the WBC recovery and WBC viability per sample.

Filter Overall Efficacy, Including Integrity Test
To evaluate the effect of the gamma irradiation process on the integrity of the Acrodisc WBC syringe filter, additional 10 gamma irradiated and 10 non-gamma irradiated filter units were tested. Testing includes a visual inspection, flow rate, bubble point and burst pressure tests, which were designed to verify the device membrane and hardware integrity. All testing was within expected results (see Table 2).

4. Results

Cell Viability and Recovery Testing
Figure 2 presents the results of the leukocyte depletion from the filtrate sample. These results confirm the ability of the Acrodisc WBC syringe filter to separate the WBC from whole blood samples, since leukodepletion was over 99% in all cases when gamma and non-gamma irradiated syringe filters were used, at both T0 and T2.

Figure 2
Leukocyte depletion (%) values from whole blood samples after filtration with the newly designed Acrodisc WBC syringe filter. Values represent the average calculated from the 20 replicates tested under each condition.

The results from the cell recovery test depicted in Figure 3, show that the Acrodisc WBC syringe filter units were able to recover WBC in the expected range of approximately 60%. Statistically similar values were obtained between the control and the γ-irradiated Acrodisc WBC syringe filter units, and also between the T0 and T2 samples.
Figure 3
WBC % recovery values using the new Acrodisc WBC syringe filter values represent the average calculated from the 20 replicates tested under each condition.

As shown in Figure 4., the viability of the WBC’s before (whole blood sample, ■) and after the filtration process (WBC recovery sample, □), yielded similar results of approximately 80%. This comparability remained constant between samples filtered with control and γ-irradiated Acrodisc WBC syringe filter units at time 0 (T0) and after the 2 year accelerated aging (T2).

Figure 4
WBC viability (%) from whole blood samples (■) and from the recovery samples after using the new Acrodisc WBC syringe filter (□). Values represent the average calculated from the 20 replicates tested under each condition.
Table 1
Results of filtration time, WBC recovery and viability analysis of whole blood samples before and after filtration with non-gamma (control) and gamma irradiated Acrodisc WBC syringe filters. Cell recovery and viability results correspond to flow cytometry analysis. Values represent the average calculated from the 20 replicates tested under each condition.

<table>
<thead>
<tr>
<th>Cell Viability Summary</th>
<th>T0</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>γ-irradiated</td>
</tr>
<tr>
<td>Average blood filtration time (min:sec)</td>
<td>11:11</td>
<td>10:46</td>
</tr>
<tr>
<td>WBC viability before filtration (%)</td>
<td>94.0</td>
<td>93.5</td>
</tr>
<tr>
<td>WBC viability after filtration (%)</td>
<td>83.9 ± 9.5</td>
<td>76.9 ± 6.8</td>
</tr>
<tr>
<td>WBC recovery (%)</td>
<td>57.4 ± 19.7</td>
<td>57.8 ± 7.8</td>
</tr>
</tbody>
</table>

a. Integrity Test results
Filter performance pre and post gamma irradiation results are detailed in Table 2. All samples met the criteria set out in the integrity testing parameters.

Table 2
Results from the integrity test (IT) performed on gamma- and non-gamma irradiated Acrodisc WBC syringe filters at time 0 (T0) and two years accelerated aging (T2). Results from non-gamma irradiated filters were set as a control. Values represent the average calculated from the ten replicates tested under each condition.

<table>
<thead>
<tr>
<th>IT Summary</th>
<th>T0</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>γ-irradiated</td>
</tr>
<tr>
<td>Mean flow rate (ml/min)</td>
<td>750.5 ± 33.4</td>
<td>784.3 ± 44.2</td>
</tr>
<tr>
<td>Mean bubble point (in H₂O)</td>
<td>77.6 ± 7.15</td>
<td>79.0 ± 6.7</td>
</tr>
<tr>
<td>Mean burst pressure (psi)</td>
<td>207.7 ± 17.9</td>
<td>156.0 ± 23.5</td>
</tr>
<tr>
<td>Overall pass/fail</td>
<td>Pass</td>
<td>Pass</td>
</tr>
</tbody>
</table>
5. Conclusions

The Acrodisc WBC syringe filter efficiently and quickly separates leukocytes from human whole blood samples. The WBC depletion is over 99% in the leukocyte depleted sample, while the average WBC recovery was 60% and the WBC viability average was 80%.

Note: viscosity variability among blood samples was observed. This variability could have been due to individual biological factors from the donors, such as fat content and high WBC counts, which can affect the filtration rate. Therefore, for a more accurate approach results before and after filtration were compared.

Today’s demanding laboratory environment requires new options to accommodate testing needs. The ability to process large number of samples quickly under laboratory conditions requires an optimized product design for leukocyte recovery or depletion. The newly designed sterile, Acrodisc WBC syringe filter from Pall Laboratory fulfills that need by facilitating the backwash-step with the new female leur lock (FLL) outlet. This product is now provided sterile by gamma irradiation and individually blister packed. Results from this scientific application note prove that the performance of the new sterile Acrodisc WBC syringe filters were statistically the same compared to the non-gamma version. This was proven by achieving comparable WBC recovery and viability results throughout the entire product shelf life.

6. References