HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank		
Document:	Internal Policy and Procedure		
Title:	Counting Residual White Cells In Leukocyte-Reduced Blood and Components		
Applies To:	All Blood Bank Staff		
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1. PURPOSE:

1.1 To estimate the efficiency of leukoreduction filter.

2. DEFINITONS:

2.1 N/A

3. POLICY:

3.1 For Leukocyte-reduced units (but not apheresis units), 1% of the quarterly production, but not less than 12 units every three months, are subjected to quality control testing.

3.2 All tested (LR-RBC) units have a RBC recovery rate of more than 85% and a residual WBC count of less than $5X10^6$ WBC/unit in all subjected units.

3.3 All tested LR-PC units have a platelets recovery rate of more than 85% and a residual WBC count of less than $8.3X10^5$ WBC/unit

3.4 Counting residual white cells in leukocyte-reduced blood and components is performed using fresh samples (within 48 hours after collection) or per the manufacturer's directions for the cell counting methodology.

4. PROCEDURE:

4.1 **Manual method:**

4.1.1 Principle:

4.1.1.1 The residual white-cell content of leukocyte-reduced (LR) whole blood and components can be determined using a large-volume hemocytometer.

4.1.1.2 For red cell containing components, the red cells (in the aliquot to be counted) are first lysed.

4.1.1.3 Accuracy of counting is improved by examining a larger volume of minimally diluted specimen, compared with standard counting techniques.

4.1.1.4 White cells deteriorate during refrigerated storage; counts on stored blood or red cell components may give inaccurate results. Therefore, counting residual white cells in leukocyte-reduced blood and components is performed using fresh samples.

4.1.2 Materials:

4.1.2.1 Hemocytometer chamber with 50-µL counting volume (e.g. Nageotte Brite Line Chamber, Biotrans GmbH, Dreieich, Germany).

4.1.2.2 Crystal violet stain (e.g. 0.01% Turk's solution) is used to stain the leukocyte nuclei.

4.1.2.3 Red cell lysing agent (e.g., Zapoglobin, Coulter Electronics, Hialeah, FL), for red Cell containing components only.

4.1.2.4 Pipetter (40 µL and 100 µL) with disposable tips.

4.1.2.5 Talc-free gloves, clean plastic test tubes, plastic petri dish, and filter paper.

4.1.2.6 Light microscope with 10x ocular lens and 20x objective.

4.1.2.7 Worksheet for recording results.

4.1.3 Samples from fresh units:

4.1.3.1 Strip the attached tubing at least four times, mixing the contents of the tubing with the contents of the bag, to ensure that the contents of the tubing accurately represent the entire contents of the bag.

4.1.3.2 Seal a 5- to 8-cm (2- to 3-inch) segment distal to the collection bag. There should be approximately 2 mL of fluid in the segment. Double-seal the end of the tubing next to the component bag and detach the segment.

4.1.3.3 Empty the contents of the segment into a suitably labeled tube.

4.1.4 Procedure: Using Nageotte hemocytometry:

4.1.4.1 Dilute and stain LR blood and component samples as follows:

4.1.4.1.1 For red cell containing components:

4.1.4.1.1.1 Pipette 40 µL of lysing agent into a clean test tube.

4.1.4.1.1.2 Place a representative sample of the component to be tested in a clean test tube. The hematocrit of the sample to be tested should not exceed 60%.

4.1.4.1.1.3 Pipette 100 µL of the sample into the tube containing 40 µL of lysing agent. Rinse the pipette several times to mix the two fluids, until the pipette tip is no longer coated with intact red cells.

4.1.4.1.1.4 Pipette 360 µL of 0.01% Turk's solution into the mixture, and mix fluids by pipetting up and down several times. The final volume is now 500µL.

4.1.4.1.2 For platelets:

4.1.4.1.2.1 Place a representative sample of the platelet in a clean test tube.

4.1.4.1.2.2 Pipette 100 µL of the platelet sample into a clean test tube.

4.1.4.1.2.3 Pipette 400 µL of 0.01% of Turk's solution into the 100 µL of platelets, and mix fluids by pipetting up and down several times. The final volume is now 500 µL.

4.1.4.2 Fit the hemocytometer with a cover slip; using a pipette, load the mixture until the counting area is completely covered but not overflowing.

4.1.4.3 Cover the hemocytometer with a moist lid to prevent evaporation (a plastic petri dish into which a piece of damp filter paper has been placed works well), and let it rest undisturbed for 10 to 15 minutes to allow the white cells to settle in the counting area of the chamber.

4.1.4.4 Remove the moist lid, place the hemocytometer on the microscope and using a 20x objective, count the white cells present in the entire 50µL volume of the counting chamber. White cells appear as intact cells that are refractile with a blue-grey color.

4.1.4.5 White cell concentration:

4.1.4.5.1 leukocytes/µL = (cells counted/50) × 5 where 50 µL is the volume counted and 5 is the dilution factor resulting from the addition of lysing agent and Turk's solution.

4.2 **Flow cytometry method:**

4.2.1 The residual white-cell content of leukocyte- reduced (LR) whole blood and components can be determined using flowcytometric technique. Some blood cell counter machines perform cell-counting analysis based on flow cytometry method.

4.2.2 No flowcytometric technique-based machines are available in MCH.

4.3 **Calculate and record results:**

4.3.1 Total white cell content of the LR component:
Leukocytes/component = leukocytes/µL × 1000 × volume (in mL) of the component.

4.3.2 Record the component's identity, the date obtained, and the identity of the person performing the testing.

4.4 **Accepted value:**

4.4.1 For Leukocyte-Reduced Red Blood Cells (LR-RBC) units:
4.4.1.1 All tested LR units must have a residual WBC count of less than $5X10^6$ WBC/unit.
4.4.2 For Leukocyte-Reduced random Platelet concentrates (LR-PC) units:
4.4.2.1 All tested LR units must have a residual WBC count of less than $8.3X10^5$WBC/ unit.
4.4.3 Corrective action:
4.4.3.1 Report to the supplier if leukocytic count is high.

4.5 **Notes:**

4.5.1 Use of talc-free gloves is recommended because talc particles, that contaminate the counting chamber, can be misread as white cells.
4.5.2 Filtration of the Turk's solution (0.22 micron) is recommended if the counting chamber demonstrates a large amount of particulates.
4.5.3 Nageotte hemocytometry counting technique is not known to be accurate at concentrations lower than 1white cell/µL.

5. MATERIALS AND EQUIPMENT:

5.1 **Forms and Records:**
5.1.1 LR-blood component QC form
5.2 **Materials:**
5.2.1 As mentioned in 4.1.2.

6. RESPONSIBILITIES:

6.1 Blood bank technician / specialist to follow the detailed procedure and to send the samples to the hematology unit to be tested.
6.2 Hematology unit staff to test for leukocytic count.

7. APPENDICES:

7.1 N/A

8. REFERENCES:

8.1 The Unified Practical Procedure Manual For Blood Banks In The Arab Countries, 1434-2013.
8.2 The Standard Policy For Blood Banks In The Kingdom Of Saudi Arabia, 1st edition, 1435-2014
8.3 National Standards For Clinical laboratories and Blood Banks, 1st edition, 2015.
8.4 AABB Technical manual, 18th edition, 2014.
8.5 AABB Standards for Blood Banks and Transfusion Services, 30th edition, 2016.
8.6 Mollison's Blood Transfusion in Clinical Medicine; 12th edition, 2014.
8.7 Modern Blood Banking & Transfusion Practices, 6th edition, 2012.
8.8 U.S. Department of Health and Human Services; Food and Drug Administration (FDA), September 2012: Guidance for Industry; Pre-Storage Leukocyte Reduction of Whole Blood and Blood Components Intended for Transfusion.
8.9 Good Manufacturing Practice for Blood Establishments, Version 2.0, May 2019, Saudi FDA

9. APPROVALS:

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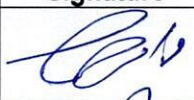
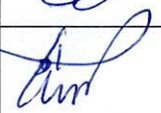
Prepared by:	Dr. Mohammed Amer	Blood Bank Physician		January 06, 2025
Reviewed by:	Dr. Kawther M. Abdou	Consultant & Lab. Medical Director		January 08, 2025
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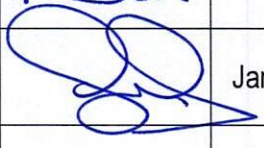
Reviewed by:	Mr. Abdulelah Ayed Al Mutairi	QM&PS Director		January 12, 2025
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