

Date: May 29, 1996

From: Director, Center for Biologics Evaluation and Research

Subject: Recommendations and Licensure Requirements for Leukocyte-Reduced Blood Products

To: All Registered Blood Establishments

I. INTRODUCTION

During the past 10 years, leukocyte-reduced blood and blood products have played an increasing role in the management of a variety of transfusion-related adverse reactions and diseases. In the United States, approximately 20% of the blood products administered are supplied or infused as leukocyte-reduced products.¹

Recent advances in leukocyte reduction technology have contributed to the ease and effectiveness of removing leukocytes from blood and blood products including Whole Blood, Red Blood Cells (RBCs), Platelets, and Platelets, Pheresis. These developments have also generated the need for guidance as questions have arisen related to terminology, quality control, product standards, efficacy claims by manufacturers, optimal conditions for leukocyte reduction, and the clinical need or benefit of using leukocyte-reduced products.

This memorandum provides general recommendations and explains licensure requirements in order to assist blood establishments in preparing leukocyte-reduced blood and blood products by methods consistent with current industry standards and practices and to facilitate licensure of these products. The document also serves as a basis for future discussions with blood establishments, manufacturers, researchers, and other interested parties.

With developments in leukocyte reduction technology ongoing, practices and standards are subject to change based on additional research data. Accordingly, the recommendations in this memorandum are evolving and are intended as guidance only. This memorandum does not bind the agency or create any rights, privileges, or benefits for, or in, any person. However, it does represent the agency's current thinking with regard to preparing leukocyte reduced blood products in blood establishments.

II. BACKGROUND

Recently, filtration and improved cytopheresis technologies without secondary processing have been increasingly used for the preparation of leukocyte-reduced blood products.

Based on the current standard of practice in transfusion medicine, many physicians use leukocyte-reduced blood products for a variety of clinical indications. Blood products subjected only to leukocyte reduction techniques have been implicated in cases of transfusion-associated graft-versus-host disease (TA-GVHD), and therefore, leukocyte reduction does not always prevent TA-GVHD.² Gamma irradiation of blood products containing viable lymphocytes continues to be the most appropriate approach to the prevention of TA-GVHD.³

III. RECOMMENDATIONS FOR MANUFACTURING

All leukocyte-reduced blood products should be manufactured and processed following applicable current Good Manufacturing Practices (cGMPs) codified in Title 21, Code of Federal Regulations (21 CFR), Parts 600 through 680 and Parts 210 and 211. Leukocyte filters used for manufacturing are usually product-specific and are not interchangeable. In addition, blood products that are leukocyte-reduced by filtration as part of manufacture (prestorage leukocyte reduction) should continue to be transfused with an administration set containing a standard blood filter.

Leukocyte-reduced products may be prepared in blood establishments after blood collection and prior to storage. Although the optimal time and temperature for leukocyte reduction has not been established, several researchers suggest that prestorage leukocyte removal may have several advantages over filtration immediately prior to transfusion. Benefits of prestorage leukocyte reduction are reported to be fewer toxic and immunogenic cell products, possible elimination of the immunomodulating effects of blood transfusion on tumor growth enhancement, lower levels of pro-inflammatory cytokines and partial removal of microbial contaminants.⁴⁻¹⁴ Furthermore, when leukocyte reduction is performed in blood establishments rather than at the bedside, product quality control may be standardized, and inventories of leukocyte-reduced blood products may be maintained.

A. Prestorage Preparation of Leukocyte-Reduced Blood Products

1. Closed Systems

The closed system can be achieved by apparatus that includes, but is not limited to:

- (a) manufacturing with an in-line filter included in the blood bag collection set;
- (b) attaching a leukocyte reduction filter to a blood component container, collection set or a cytopheresis collection set with a Food and Drug Administration (FDA)-cleared Sterile Connecting Device (STCD);¹⁵
- (c) using a functionally closed system such as a platelet collection system that is totally closed at the point of manufacture except for preconnected solutions and /or preconnected FDA approved bacteriostatic filters on spike and port

connections; or (d) preparing a product such as Platelets, Pheresis with cytopheresis equipment that does not require the addition of a filter system in order to produce a leukocyte reduced product. If the closed system is maintained, the expiration date of the final leukocyte reduced product remains the same as that of the original product.

2. Open Systems

The open system involves attaching a filter to one of the ports on the blood bag collection set using aseptic technique. After entering the container in this manner, the red blood cells have a 24-hour expiration date when stored at 1-6^o C. Platelets that are pooled and then filtered have an expiration date of 4 hours after entering the containers to pool the product.¹⁶

3. Platelets, Pheresis

The expiration date of the leukocyte-reduced platelet product will depend upon whether the platelet product was collected in an open system or a functionally closed system as described above. External needles or satellite bags may only be attached to these systems using an FDA-cleared STCD. Platelets, Pheresis collected in an open system expire 24 hours from the termination of the procedure. Platelets, Pheresis collected in an approved 5 day functionally closed system expire on midnight of the fifth day.¹⁷

B. Standard Operating Procedures (SOPs)

The SOPs for manufacturing leukocyte-reduced blood products should include the following information:

1. Name and manufacturer of the equipment used for leukocyte reduction. Prestorage filters or direct cytopheresis equipment should be FDA-cleared for the specific product, manufactured and intended for use in prestorage leukocyte reduction. Filters intended for bedside filtration should not be used for pre-storage filtration.
2. Leukocyte reduction procedures consistent with manufacturers' instructions including quality control procedures. Methods of investigation of quality control failures.
3. Time period when leukocyte reduction is performed, i.e., during the initial 8-hour room temperature hold or after refrigeration consistent with manufacturers' instructions.
4. A description of all procedures related to the use of STCDs when these devices are used in leukocyte reduction.
5. Procedures for handling units that do not meet the criteria for labeling the product as leukocyte-

reduced, following the leukocyte reduction procedure.

IV. LABELING

Previous labeling for leukocyte-reduced products included the terms, "Leukocytes Removed," "Leukocyte Poor," and "Leukocyte Depleted" which are no longer considered appropriate by FDA. The following labeling reflects the current terminology approved by the FDA for leukocyte-reduced products.

A. Whole Blood

Whole blood products prepared by a method known to leave a residual leukocyte count of less than 5×10^6 per container and retain a minimum of 85% of the original product should be labeled, "Whole Blood, Leukocytes Reduced," including appropriate modifiers, e.g., CPD Whole Blood, Leukocytes Reduced, etc. The product code formerly used for "Whole Blood, Leukocytes Removed" should be used for "Whole Blood, Leukocytes Reduced."

B. Red Blood Cells

Red blood cell products prepared by a method known to leave a residual leukocyte count of less than 5×10^6 per container and retain a minimum of 85% of the original product should be labeled, "Red Blood Cells, Leukocytes Reduced", including appropriate modifiers, e.g., CPD Red Blood Cells, Leukocytes, Reduced, etc. The product codes formerly used for "Red Blood Cells, Leukocytes Removed" should be used for "Red Blood Cells, Leukocytes Reduced."

C. Platelets

Platelet products prepared from whole blood by a method known to leave a residual leukocyte count of less than 8.3×10^5 per individual container and retain a minimum of 85% of the unpooled product should be labeled "Platelets, Leukocytes Reduced." Leukocyte reduced pooled platelet concentrates are not considered products licensable by the FDA.

D. Platelets, Pheresis

Platelets, Pheresis products prepared by cytapheresis without secondary processing (such as filtration) having a residual leukocyte count of less than 5×10^6 per container should be labeled, "Platelets, Pheresis, Leukocytes Reduced." Platelet Pheresis products, leukocyte reduced by filtration with a residual leukocyte count of less than 5×10^6 per container and retaining a minimum of 85% of the original product should be labeled, "Platelets, Pheresis, Leukocytes Reduced."

Products further modified from the original products listed above, e.g., Irradiated, Divided, etc. should put the additional words after "Leukocytes Reduced" on the label to indicate the modification.

E. Circular of Information

The Circular of Information constitutes labeling for blood products and contains indications, contraindications, side effects, hazards, dosage, and administration of leukocyte-reduced products.¹⁸ This information is regularly updated by the blood industry and is subject to approval by the FDA. Currently, blood establishments should add an addendum to the Circular of Information to meet the standards defined in this memorandum. Licensed establishments should submit this information with their product license application (PLA).

V. QUALITY CONTROL PROCEDURES

Process validation is a requirement of the Current Good Manufacturing Practices Regulations for Finished Pharmaceuticals, 21 CFR Parts 210 and 211, mentioned above, and of the Good Manufacturing Practice Regulations for Medical Devices, 21 CFR 820, and therefore, is applicable to the manufacture of pharmaceuticals and medical devices.¹⁹ Manufacturers of leukocyte reduced products should perform extensive quality control (QC) procedures to assure that leukocyte reduction equipment meets specifications and labeling claims.

In addition, manufacturers should continue to generate data to support the claims that their production process remains stable and consistent over time and any variation does not exceed product specifications.

It is essential that the validation program is documented and that the documentation is properly maintained at the manufacturing site.¹⁹

Current Good Manufacturing Practices (cGMPs) for blood and blood product manufacturers require that end users develop and follow written QC procedures to verify that the technology has been applied correctly and that finished blood products meet established criteria. QC procedures should include periodic monitoring of residual leukocyte counts using validated methods, described below in section C, that have the appropriate sensitivity to consistently detect the limits of expected numbers of residual leukocytes and to accurately measure the quantity of the original product remaining after leukocyte reduction.

A. Samples

A representative sample should be collected from leukocyte reduced products by using an FDA-

cleared STCD to attach additional tubing or a suitable storage container, or from freshly stripped and filled tubing segments. Device manufacturer's directions or validated sample collection procedures should be followed. Samples should be handled, prepared and processed without delay according to the requirements of the counting method to ensure that a true and representative count is obtained.

B. Sampling Plan

Each blood establishment should develop an appropriate sampling plan for testing leukocyte-reduced products. Establishments may sample and count the total required number of donor units per month from units collected within the previous 30 days. The sampling plan should include testing a minimum of 1% of the establishment's monthly production of each leukocyte-reduced blood product (Whole Blood, Red Blood Cells, Platelets, or Platelets, Pheresis), or for establishments producing under 400 units per month, 4 units per month of each leukocyte-reduced blood product.²⁰ All units tested (100%) should meet the standard as described in the section on labeling for use as a leukocyte-reduced blood product. Units which fail to meet the minimum criteria may be used for transfusion provided that a closed system is maintained and the products are not labeled as leukocytes reduced. Products should not be refiltered routinely as this may cause additional loss of the original product intended for transfusion. The sampling plan should provide a level of confidence that assures a high probability of detecting errors in application of leukocyte reduction technology. The units selected for counting should be selected at random to adequately represent the larger population of units from which they were selected.²¹

C. Counting Methods

Leukocyte reduced blood products should contain less than 5×10^6 residual leukocytes, except for platelet concentrates which should contain less than 8.3×10^5 residual leukocytes. Special methods of cell counting are required to detect such low numbers of leukocytes. Automated hematology cell counting devices may not accurately determine the residual leukocytes in leukocyte reduced products. The counting methods that should be used for evaluating low numbers of residual leukocytes are as follows:

1. Manual methods:

Methods using a Nageotte counting chamber and Turk's or Plaxan's staining solutions alone or in conjunction with Zap-oglobin[®], or centrifugation are comparable in sensitivity and reliability for counting less than 5 leukocytes/uL.²²⁻²⁸

2. Automated method:

Flow cytometry, using propidium iodide alone or combined with thiazole orange, compares

favorably in sensitivity to the Nageotte chamber method and provides the advantages of automation and the ability to determine phenotypes of residual leukocytes which may be of interest to clinicians.^{25,29}

Other validated methods may also be acceptable provided that they achieve the desired level of sensitivity for counting the residual leukocytes.

D. Expected Results

Leukocyte reduced products meet the standard for leukocyte reduction when less than 5×10^6 leukocytes per container (unit) remain after preparation and a minimum of 85% of Whole Blood or Red Blood Cells is retained. Individual Platelet concentrates meet the standard when less than 8.3×10^5 leukocytes per container remain after preparation and 85% of the product is retained. Platelets, Pheresis products, leukocyte reduced by filtration meet the standard when less than 5×10^6 leukocytes per container remain after preparation and 85% of the product is retained. Platelet Pheresis products prepared by cytopheresis without secondary processing (such as filtration) meet the standard when less than 5×10^6 leukocytes per container remain after preparation. The counting method validation should include consideration of the time between blood sampling and counting. The units tested should meet the established criteria defined in the sampling plan so that product specifications meet the requirements for safety and effectiveness in accordance with the product label as a leukocyte reduced product. Standard operating procedures (SOPs) should include procedures for handling units that fail QC testing and for initiating an investigation of the failures so that corrective action can be instituted. The results of the investigation and corrective action should be documented. Units that fail QC testing but retain a minimum of 85% of the original product may be used as non-leukocyte reduced products provided a closed system is maintained and they are not labeled as leukocyte reduced. They should not be routinely reprocessed to remove leukocytes. If QC testing and evaluation of the leukocyte reduction process indicates that the process is no longer stable and/or not capable of meeting the criteria for leukocyte reduced products, blood establishments should examine the preparation and processing of leukocyte reduced products and proficiency in the use of the counting method. If necessary, technical advice should be sought from the cytopheresis equipment or filter manufacturer.

VI. TRAINING/CONTINUING EDUCATION

Staff preparing leukocyte reduced products should be well-trained to perform their duties and retrained, as needed, on a continuing basis. Training should include the following:

1. General information about product handling and consequences to recipients if the products do not meet the specifications.
2. Preparation methods.
3. Collection, storage, preparation and stability of quality control samples.

4. Labeling procedures.
5. Quality control testing procedures.
6. Equipment preventive maintenance.
7. Routine in-service education.

VII. RECORDS

Records must be maintained according to 21 CFR 606.160 and reviewed to assure that an accurate history of all work performed, training, preventive maintenance, quality assurance, and continuing education are maintained. Additionally, records should include a list of filters used; their identification or lot numbers; dates received; dates of expiration; filter manufacturers' package inserts and validation data for the leukocyte counting method used.

Incident logs and preventive maintenance records should be kept for all cytapheresis equipment.

VIII. LICENSURE REQUIREMENTS

Pursuant to 21 CFR, Part 607, any establishments routinely manufacturing leukocyte reduced blood products must register (if not already registered) with the FDA within 5 days of initiating this activity and annually thereafter using Form FDA 2830. In addition, any establishments shipping these products in interstate commerce must be licensed in accordance with Section 351 of the Public Health Service Act. FDA registration is not required for products that will be leukocyte reduced at the bedside.

Licensed blood establishments should request and wait for approval of a supplement to their applicable product licenses prior to implementation. The following forms and information should be submitted to the FDA at the address provided in Part IX:

1. Whole Blood, Form FDA 3098; or Red Blood Cells, Form FDA 3098a; for Platelets, Form FDA 3098c; and, if applicable, for Platelets, Pheresis, Form FDA 3098e.
2. SOPs for the preparation of products including the information described above in Part IIIB.
3. Cell counting method and where it is performed. If not performed at the blood establishment, a laboratory certified by the Health Care Financing Administration (HCFA) may be used. A written agreement should be on file permitting authorized FDA inspectors to inspect the testing laboratory (Form FDA 3098c, section 6c).
4. Two months of QC data for each product demonstrating the adequacy of the method used for preparing leukocyte-reduced products by providing the residual leukocyte counts and hematocrit or platelet counts of the product remaining after leukocyte reduction. Documentation of monthly QC should be available for review at the time of FDA inspections.

5. Product labels consistent with these recommendations and Draft Revision of FDA Guideline for the Uniform Labeling of Blood and Blood Products, 1989. The product codes listed for Whole blood and Red Blood Cells, Leukocytes Removed by Filtration may be used for Whole Blood and Red Blood Cells, Leukocytes Reduced.
6. Evidence of supervisory review of production and QC records.

Until Forms FDA 3098, 3098a, 3098c, and 3098e are updated, the licensure information requested and quality control recommendations stated in this memorandum supersede those described on the forms for leukocyte reduced products.

Licensed establishments with approved supplements to manufacture Red Blood Cells, Leukocytes Removed by Filtration using FDA-cleared filters, only need to submit labeling to reflect the labeling changes discussed in this memorandum. Licensed establishments changing to a different FDA-cleared filter, including those requiring the use of an STCD and using the same anticoagulant, do not have to notify the FDA.

IX. ADDITIONAL INFORMATION

Questions concerning whole blood and red blood cells that are leukocyte reduced may be submitted to the following address:

Director, Division of Blood Applications (HFM-370)
Food and Drug Administration
Center for Biologics Evaluation and Research
c/o Document Control Center (HFM-99)
1401 Rockville Pike, Suite 200N
Rockville, MD 20852-1448
Voice telephone number: (301) 827-3543,
Fax number: (301) 827-2857

Questions concerning platelets and platelet pheresis products that are leukocyte reduced may be addressed to the Director, Division of Hematology, (HFM-330) at the above address. Voice telephone number (301) 496-4396, FAX number (301) 402-2780.

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