Guidance for Industry

Pre-Storage Leukocyte Reduction of Whole Blood and Blood Components Intended for Transfusion

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I. INTRODUCTION

We, FDA, are issuing this guidance document to provide you, blood establishments, with recommendations for pre-storage leukocyte reduction of Whole Blood and blood components intended for transfusion, including recommendations for validation and quality control monitoring of the leukocyte reduction process. We also provide information to licensed blood establishments for submitting biologics license application supplements to include leukocytes reduced components. This guidance applies to Whole Blood, Red Blood Cells, Plasma, and Platelets[1] manufactured from Whole Blood or collected by automated methods[2]. This guidance document finalizes the draft guidance of the same title dated January 2011 and supersedes the FDA memorandum issued on May 29, 1996, entitled "Recommendations and Licensure Requirements for Leukocyte-Reduced Blood Products."

We support the use of leukocytes reduced blood and blood components for specific indications (see section II.B) and seek to streamline the licensing procedure for leukocytes reduced blood components to assist blood establishments in making pre-storage leukocytes reduced blood components more widely available.

Although there have been reports of adverse events associated with leukocyte reduction by filtration (Refs. 1 and 2), advances in blood cell separation technology generally enable the safe reduction of leukocytes. We believe that increased availability of pre-storage leukocytes reduced blood components for non-targeted recipients will support the treatment of recipients who may

[1] The guidance document entitled "Guidance for Industry and FDA Review Staff: Collection of Platelets by Automated Methods" dated December 2007 contains our recommendations for validating; quality assurance and monitoring; labeling and licensure for leukocyte reduction of Platelet, Pheresis products. http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm073382.htm.

[2] The guidance document entitled "Guidance for Industry: Recommendations for Collecting Red Blood Cells by Automated Apheresis Methods" Technical Correction dated February 2001. http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm076756.htm.

benefit from receiving leukocytes reduced components but have not been identified as falling within the indications identified in section II.B. We suggest that consideration should be given to making leukocytes reduced blood components more widely available.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

A. Changes from FDA Memorandum, "Recommendations and Licensure Requirements for Leukocyte-Reduced Blood Products," May 29, 1996

In a May 29, 1996 memorandum, we issued recommendations on leukocyte reduction, a manufacturing step performed under controlled and monitored laboratory conditions. Under those recommendations, leukocytes reduced components were to contain $< 5.0 \times 10^6$ residual white blood cells (WBCs) per each Whole Blood, Red Blood Cells or Platelets, Pheresis collection, and $< 8.3 \times 10^5$ residual WBCs per each Platelets derived from Whole Blood, with at least an 85 percent recovery of the original component. The memorandum was consistent with the outcome of a public workshop held in March 1995 on leukocytes reduced products. This guidance document maintains those standards for residual WBCs and modifies the 1996 recommendations as follows:

1. Statistical methods for validation and quality control monitoring of the leukocyte reduction process are provided. Consideration is now given to the distinction between process and non-process failures.

2. This document discusses certain donor specific traits which may affect leukocyte reduction processes. Observations have identified a number of donor-specific factors (such as abnormal polymerization of hemoglobin S (HbS)) during the filtration process (Refs. 3 through 7) that may result in filter blockage or inadequate leukocyte reduction. The identification of sickle cell trait or other factors associated with incomplete filtration for a given donor is an important adjunct to overall process control. We encourage blood establishments to reduce component loss by identifying donors whose blood fails to filter successfully and to divert subsequent donations from such donors for uses other than leukocytes reduced transfusable Whole Blood and blood components. Testing of donors for HbS may be appropriate.

3. We are also providing an option for supplemental labeling of blood components (not applicable to Whole Blood derived Platelets) when the residual WBC count of an individual component has been determined by direct count to be $< 1.0 \times 10^6$. A supplemental label in the form of a tie-tag may be affixed to the container stating: "The residual white blood cell count

of this component has been determined to be < 1.0×10^6." We make this recommendation in response to comments requesting more specific labeling for leukocytes reduced products with a lower WBC count.

4. We recommend that Red Blood Cell recovery should be calculated by comparison of the post-filtration to pre-filtration volume x hematocrit, weight x hematocrit, or weight unless otherwise instructed in the manufacturer's direction for use.

B. Established Benefits of Pre-Storage Leukocyte Reduction

Leukocytes reduced Whole Blood and blood components have been shown to reduce the following:

- recurrent febrile, non-hemolytic transfusion reaction (i.e., patients with a history of two or more febrile reactions to transfusion) (Refs. 8 through 12);
- alloimmunization to leukocyte antigens that may complicate care of patients who undergo transplantation or chronic transfusion therapy (e.g., patients with aplastic anemia or hematologic malignancies); and
- transmission of cytomegalovirus (CMV) to patients at increased risk of CMV disease (e.g., chemotherapy recipients for whom severe neutropenia is expected, recipients of hematopoietic progenitor cell replacement therapy, CMV seronegative recipients of CMV seronegative solid organ grafts, and low birth weight premature infants) (Ref. 13).

We believe it is advantageous to provide leukocytes reduced components to reduce such adverse events. In addition, we believe it is advantageous to provide pre-storage leukocytes reduced components in preference to bedside filtered blood components based on quality and safety considerations (Ref. 14). Routine use of leukocytes reduced blood components further protects patients who have increased risk, but whose conditions have not yet been identified.

C. Potential Benefits of Pre-Storage Leukocyte Reduction

Potential, but not established, benefits of leukocyte reduction include reduction of:

- transfusion-associated immunomodulation (Ref. 15);
- bacterial overgrowth (Refs. 8 and 16);
- viral reactivation (Refs. 8 and 16);
- reperfusion injury following cardiopulmonary bypass (Ref. 16);
- red blood cell and platelet storage lesions (Ref. 17);
- transfusion-transmitted *Leishmania* infection (Ref. 18); and
- transfusion-transmitted variant Creutzfeldt-Jakob disease (vCJD) (Ref. 19).

Limited scientific data have suggested the possibility that leukocyte reduction may be a useful measure against the risk of transfusion-transmitted vCJD (Refs. 19 through 23).

Some international blood authorities have implemented or are considering the implementation of a regulatory requirement to leukocyte reduce all Red Blood Cells and Platelets intended for transfusion (universal leukocyte reduction), as well as plasma for further manufacturing to reduce the risk of vCJD transmission.

Similarly, the potential to reduce infection by pathogens that are primarily WBC associated may extend beyond CMV (Refs. 11 and 12) to include other known and unknown agents potentially transmitted by WBCs (Ref. 19).

Leukocyte reduction is not considered appropriate for the prevention of transfusion-associated graft-versus-host disease. At this time, irradiation of blood products is the only definitive method available to prevent this serious and often fatal transfusion outcome in patients at risk (Ref. 24).

D. Safety Concerns Related to Bedside Leukocyte Reduction Filtration

Bedside filtration remains available as a leukocyte reduction method to physicians prescribing transfusion therapy. As a post-storage procedure, however, bedside filtration has been associated with precipitous hypotension in the transfusion recipient, an infrequent yet serious adverse effect not associated with pre-storage leukocyte reduction (Ref. 25). Patients on medications that inhibit the angiotensin converting enzyme (ACE inhibitors) appear to be particularly susceptible. Pre-storage leukocyte reduction allows the leukocyte reduction process to be monitored under controlled conditions that assure component purity, consistency, and safety and is therefore generally preferable to bedside filtration (Refs. 25 through 27).

E. Definitions

For the purposes of the terms used in this guidance, the following definitions apply:

Automated Blood Cell Separator (ABCS): A device that uses a centrifugal or filtration separation principle to automatically withdraw Whole Blood from a donor, separate the Whole Blood into blood components, collect one or more of the blood components and return to the donor the remainder of the Whole Blood and blood components. The ABCS device is intended for routine collection of blood and blood components for transfusion or further manufacturing use.

Non-process failure: Failure of a leukocyte reduction process due to a non-controllable parameter (i.e., donor specific characteristic such as HbS). Note: In your sampling plan for validation or quality control (QC) testing, you may exclude (and replace) non-process failures.

Percent recovery (of the original component): Ratio of the post-filtration to the pre-filtration content of the component expressed as a percent.

- **Red Blood Cells/Whole Blood:** Red Blood Cell percent recovery of the original component can be determined by comparing the pre-filtration content to the post-filtration content by using any of the following methods:
  - volume x hematocrit;
  - weight x hematocrit;
  - weight: The weight method should be used in conjunction with a documented visual check to ensure that abnormal red blood cell retention has not occurred; or
  - as described by the manufacturer in the package insert.

- **Platelets:** Because of selective removal of platelets by leukocyte reduction filters, the percent recovery is to be determined by comparing the platelet yield pre-filtration to the platelet yield post-filtration (i.e., not by weight).

Process failure: Failure of a leukocyte reduction process due to an error that was avoidable if proper procedures, precautions and/or instructions were followed (i.e., not following manufacturer's directions) or a product/device defect.

Process validation: In brief, is the collection and evaluation of data from the process design stage through production. This validation establishes scientific evidence that a process is capable of consistently delivering quality product (Ref. 28).

Residual White Blood Cell (WBC) content: The number of WBCs remaining in a leukocytes reduced component, calculated by multiplying the WBC count from a sample of the component times the volume of the component.

Yield: The quantity that is actually produced during manufacturing.

III. VALIDATION OF THE LEUKOCYTE REDUCTION PROCESS

The Current Good Manufacturing Practices (cGMP) regulations described in 21 CFR Parts 210 and 211 contain the minimum requirements for methods to be used in, and the facilities or controls to be used for, the manufacture, processing, packing or holding of a drug to assure that the drug meets the safety requirements of the Federal Food, Drug, and Cosmetic Act, and has the identity and strength and meets the quality and purity characteristics that it purports or is represented to possess (21 CFR 210.1(a)). These cGMP regulations apply to Whole Blood and blood components (21 CFR 210.2(a) and 211.1(b)) and supplement the cGMP regulations for blood and blood components contained in 21 CFR Part 606. As an element of cGMP, process validation establishes "scientific evidence that a process is capable of consistently delivering quality product"[3] (Ref. 28). We recommend that establishing documentation of process validation include, but not be limited to, equipment installation qualification, validation protocol development, process operator performance qualification and product performance qualification (Ref. 28).

[3] The requirement for process control is set forth in general terms in 21 CFR 211.100.

A. Methods of Leukocyte Reduction

Whole Blood and blood components (including components collected by automated methods) intended for transfusion may be leukocyte reduced using any of the closed system or functionally closed methods as follows:

- filtration through an in-line filter integral to the blood collection set or apheresis set with or without automated filtration (e.g., press through filtration)
- filtration through a filter system attached to a blood component container using an FDA cleared sterile tubing connecting device (STCD) (Ref. 29)
- direct, in-process leukocyte reduction for certain plateletpheresis and plasmapheresis collections (i.e., by ABCS devices).

B. Equipment Installation Qualification

Section 606.60(a) (21 CFR 606.60(a)) requires that equipment be observed, standardized and calibrated on a regularly scheduled basis as prescribed in the Standard Operating Procedures Manual and must perform in the manner for which it was designed. Upon initiation of an automated leukocyte reduction method, we recommend that the equipment used for leukocyte reduction (e.g., leukocyte reduction filter, ABCS device) be qualified as described in the operator's manual or manufacturer's directions for use.

C. Validation Protocol Development

An integral element of the performance and documentation of process validation is the development of a validation protocol. You should refer to FDA's "Guidance for Industry: Process Validation: General Principles and Practices" (Ref. 28) as an outline for developing your validation protocol. We recommend that the validation protocol include, but may not be limited to:

- a description of the leukocyte reduction method, including the device, to be used
- acceptable values for the residual WBC count, including:
  - residual WBC count post leukocyte reduction for the component (Whole Blood, Red Blood Cells, Platelets, Plasma)
  - percent recovery of the original component for Whole Blood, Red Blood Cells, or Platelets when leukocytes are reduced by filtration or per the manufacturer's specifications, if different
- manufacturer's specifications for processing parameters (if given)
- failure investigation criteria
- personnel training criteria
- standard operating procedures (SOPs) for performing each element of the collection and manufacturing process
- documentation of the validation protocol criteria for all of the above.

D. Process Performance Qualification (Operator)

Each person engaged in the manufacture of leukocytes reduced components must have adequate education, training, or experience to assure their competent use of the devices involved (21 CFR 606.20(b)).

We recommend that personnel training include the successful consecutive performance, under supervision, of an appropriate number of procedures, as defined by your facility. These procedures should result in leukocytes reduced components meeting the specifications for the specific leukocyte reduction methodology being qualified.

E. Product Performance Qualification for the Leukocyte Reduction Process

Various factors may adversely influence the leukocyte reduction process, e.g., donor specific factors or improper use of an ABCS device. The objective of product performance qualification is to verify that the leukocyte reduction method conforms to the claims of the device manufacturer and the blood establishment. In addition, appropriate testing will establish confidence that the final component meets all release requirements for safety, purity, and potency (Refs. 28 through 31). All components collected during the qualification process can be released for transfusion provided that they meet minimum specifications as defined by the device manufacturer, are labeled appropriately, and are otherwise suitable.

We believe that, generally, product performance qualification only needs to be performed when the leukocyte reduction method (either by ABCS devices or filtration) is first put into use at an establishment. We note the following:

- For establishments using a single model ABCS device from only one manufacturer, all instruments available for use with that ABCS device should be included in the initial product performance qualification.
- For establishments using multiple models of ABCS devices from one or more manufacturers you should conduct full product performance qualification for each device type on all instruments available for use with that ABCS device type.
- For establishments using leukocyte reduction filters from different manufacturers, or when there is a change to a different filter manufacturer, you should conduct full product performance qualification for each filter type from each manufacturer.

Product performance qualification should include testing for the residual WBC count and percent recovery of the original component as recommended in section III.C, and testing of components processed by all trained operators. In addition, we recommend you perform residual WBC count testing within 48 hours of collection (Ref. 33) or per the manufacturer's directions for the cell counting methodology.

You must conduct an investigation of each product performance qualification failure, and when appropriate, initiate corrective action and follow-up measures (21 CFR 211.192 and 606.100(c)). There should be ongoing assurance that the process continues to perform at

the level established during the product performance qualification. Evaluating the performance of the process identifies problems and determines whether action must be taken to correct, anticipate, and prevent problems (21 CFR 211.180(e), 211.192, and 606.100(c)). We understand that some failures may occur due to conditions not resulting from a failure of the process (e.g., failure due to donor specific characteristic such as HbS).

F. Leukocyte Reduction Performance Qualification Criteria

Conformance to product standards must be assessed by a statistically valid method (see 21 CFR 211.160(b)). In the absence of a validation method (plan) provided by the manufacturer, you should develop a statistically valid plan based on 95% confidence that more than 95% of the components will meet the recommended results. One possible approach to process validation is a two-stage sampling plan based upon the binomial distribution. In this approach, an initial representative sample of components with a pre-determined sample size should be tested consecutively (validation) or randomly (QC monitoring). Identified non-process failures may be excluded from the sample size and replaced. Conformance can be demonstrated if an acceptable number of process failures are observed in this sample. In the event that there is one more process failure than permitted in the initial sample, a second sample of components may be tested consecutively. Conformance can be demonstrated if no further process failures are observed in the second sample. The size of the second sample will depend on the size of the first sample. We provide two examples to illustrate the use of this approach:

Example 1: The first stage sample is 60 consecutive collections. If no process failures are observed among these 60 components, conformance has been demonstrated and no further testing is required. If one process failure is observed among the 60 components, a second sample of 71 consecutive collections may be tested. If no further process failures are observed among these 71 components, conformance has been demonstrated and no further testing is required. If more than one process failure is observed among the first sample of 60 components, or if one or more additional process failures are observed among the second sample of 71 components, the validation has failed and no additional testing is recommended to qualify the process. A failure investigation should be undertaken. (See section III.G.)

Example 2: The first stage sample is 94 consecutive collections. If zero or one process failures are observed among these 94 components, conformance has been demonstrated and no further testing is required. If two process failures are observed among the 94 components, a second sample of 75 consecutive collections may be tested. If no further process failures are observed among these 75 components, conformance has been demonstrated and no further testing is required. If more than two process failures are observed among the first sample of 94 components, or if one or more additional process failures are observed among the second sample of 75 components, the validation has failed and no additional testing is recommended to qualify the process. A failure investigation should be undertaken. (See section III.G.)

Note that the sample sizes are pre-determined and fixed based on the required statistical criteria. For example, if you plan to test 94 samples and encounter no failure in the first 60 samples, changing your plan to 60 samples is not permitted.

In some cases, you may decide on a one-stage sampling strategy rather than the two-stage strategy described above. Based on the binomial distribution, conformance can be demonstrated by observing zero process failures in a fixed sample of 60 components, one or fewer failures in a fixed sample of 94 components, or two or fewer failures in a fixed sample of 124 components.

We recommend that the following parameters as listed in Table 1 should be assessed to assure an overall statistically-based level of product conformance:

- residual WBC content
- percent recovery of the original component ***OR*** per the manufacturer's specifications.

Table 1: Leukocyte Reduction (LR) Performance Qualification Criteria for Blood and Blood Components (other than Platelets, Pheresis[4])

Blood Component	Recommended Residual WBC Content	Recommended Minimum Post-Filtration
Whole Blood, LR	$< 5.0 \times 10^{6*}$	85% recovery*** of original RBC content
Red Blood Cells, LR	$< 5.0 \times 10^{6*}$	85% recovery*** of original RBC content
Plasma Products that are LR	$< 5.0 \times 10^{6*}$	N/A
Platelets, LR**	$< 8.3 \times 10^{5}$ per individual (i.e., unpooled) unit	85% recovery*** of original platelet yield; and a minimum of $> 5.5 \times 10^{10}$ platelets for 75% of units

*Per component.

**Multiple units of Platelets are often pooled after storage and prior to filtration, rather than filtered individually prior to storage and pooling. In this instance, the pooled and then filtered product for transfusion should contain $< 5.0 \times 10^{6}$ residual WBCs. For example, multiple units of Platelets (4-6 units) are pooled into a single platelet dose. The total (4-6 units) should contain $< 5.0 \times 10^{6}$ residual leukocytes. The same recommendation for $< 5.0 \times 10^{6}$ residual WBCs applies if platelet units are pooled before storage, whether filtered before or after storage.

***Or per the device manufacturer's specifications, if different.

Plasma components, when manufactured under cGMP, may inherently have a residual WBC content of $< 5.0 \times 10^{6}$ per unit. Currently accepted and validated cell counting procedures may need to be modified for plasma components. Plasma components (including Fresh Frozen Plasma and Cryoprecipitated Antihemophilic Factor) may be

[4] See footnote 1.

labeled "Leukocytes-Reduced" provided that manufacturing process validation as described in this section (section III) includes these components and the ABCS device is cleared to manufacture the product.

G. Investigation of Product Performance Qualification Failure

Section 211.192 (21 CFR 211.192) requires that the failure of a batch or any of its components to meet any of its specifications be thoroughly investigated. If a post-filtration residual WBC count is $\geq 5 \times 10^6$ ($\geq 8.3 \times 10^5$ for unpooled Platelets) and/or fails to meet the percent recovery of the original component during validation, you must conduct an investigation of the product performance qualification failure (21 CFR 211.192 and 606.100(c)).

You must investigate collections that fail to meet the percent Red Blood Cell recovery or Platelet recovery criteria (21 CFR 211.192 and 606.100(c)). However, the component may be released if the actual platelet yield or Red Blood Cell volume is determined to be suitable by the quality control unit and the component is labeled appropriately.

Product performance qualification failures may occur due to conditions not resulting from a failure of the process. For example, if the observed failure is investigated and found to be due to a donor-specific factor, it does not constitute a process failure and should be excluded from the performance qualification evaluation.

When a failure during product performance qualification is investigated and found to be due to an identified donor-specific factor, we encourage you to flag the donor record. Upon a subsequent occurrence of incomplete filtration or inadequate WBC removal, we encourage you to consider not using this donor for future donations of leukocytes reduced components.

H. Re-Qualification/Re-Validation

- Exceeding the allowable process failures of the product performance qualification may indicate that the process is not in control. You must investigate and take appropriate follow-up action on the source of this failure (21 CFR 211.192 and 606.100(c)) and should repeat validation.
- The manufacturer may provide re-qualification requirements for the device used in the leukocyte reduction process.

IV. QUALITY ASSURANCE AND MONITORING

Quality assurance (QA) is the sum of activities planned and performed to provide confidence that all systems and system elements that influence the quality of the component are functioning as expected (Ref. 30). When this is demonstrated, the process is considered to be in a state of control. The determination of whether a process is operating in a state of control is made by analyzing the day-to-day process, performance variability and the data for conformance with the

manufacturer's specifications. Please refer to FDA's "Guideline for Quality Assurance in Blood Establishments" (Ref. 30) for assistance in developing a QA and monitoring program. This program is distinct from the initial validation described in section III.

You must have a QC unit that has the responsibility and authority to approve or reject all components, containers, closures, in-process materials, packing materials, labeling and drug products and the authority to review production records to assure that no errors have occurred or, if errors have occurred, that they have been fully investigated (21 CFR 211.22(a)). Thus, the QC unit's responsibilities include the review of production records, and the review of complaints involving the possible failure of a product to meet its specifications (See, for example, 21 CFR 211.22, 211.192, 211.198, and 606.100(c)). In addition, your laboratory control procedures must include adequate provisions for monitoring the reliability, accuracy, precision and performance of laboratory test procedures and instruments (21 CFR 606.140(b)).

A. Component Testing

A QA program should include in-process monitoring of the manufacturing procedures and QC testing (Ref. 16).

Under 21 CFR 211.160(b), laboratory controls must include the establishment of scientifically sound and appropriate specifications, standards, sampling plans and test procedures. We will consider statistical plans that confirm a < 5% non-conformance rate with 95% confidence.

One example of a scientifically sound statistical sampling and analytic plan is based on a two-stage binomial approach. The sampling sizes described in section III.F will confirm with 95% confidence a < 5% non-conformance rate for residual WBC counts. Another example of a scientifically sound plan is based on the hypergeometric distribution (see Appendix). The hypergeometric plan is only for quality control sampling plans and cannot be used for validation. Other statistical plans may also be appropriate, such as the use of scan statistics (Ref. 32) or a lower confidence limit (e.g., for platelet yields we will consider statistical plans that confirm a < 5% non-conformance rate with 75% confidence).

As part of your QC procedures, we recommend that you:

- define a plan which representatively identifies collections to be tested for each component type (Whole Blood, Red Blood Cells, Platelets and Plasma) and ensures testing for each leukocyte reduced method (i.e., each filter type in use or each type of apheresis collection device) as detailed in Table 2;
- test the component (Whole Blood, Red Blood Cells, Plasma, Platelets);
- test for the residual WBC count within 48 hours after collection (Ref. 33), or per the manufacturer's directions for the cell counting methodology;
- test for percent recovery of the original component for Whole Blood, Red Blood Cells or Platelets or per manufacturer's directions for use (e.g., minimum Red Blood Cell volume);

- determine the volume of the collection/component on the day of QC testing, after removal of all samples;
- define procedures for investigating failures during QC, including the criteria that will categorize a failure as either a process or non-process failure. Incomplete filtration and post-filtration residual WBC counts in excess of the defined standards are to be considered as two distinct types of process failure.

Table 2: For blood and blood components (other than Platelets, Pheresis[5]), we recommend the following QC results to be acceptable:

Blood Component	Residual WBC Content	Minimum Post-Filtration
Whole Blood, LR	$< 5.0 \times 10^{6*}$	85% recovery*** of original WB content
Red Blood Cells, LR	$< 5.0 \times 10^{6*}$	85% recovery*** of original RBC content
Plasma Products, LR	$< 5.0 \times 10^{6*}$	N/A
Platelets, LR**	$< 8.3 \times 10^{5}$ per individual (i.e., unpooled) unit	85% recovery*** of original platelet yield; and a minimum of $> 5.5 \times 10^{10}$ platelets for 75% of units

*Per component.

**Multiple units of Platelets are often pooled after storage and prior to filtration, rather than filtered individually prior to storage and pooling. In this instance, the pooled and then filtered product for transfusion should contain $< 5.0 \times 10^{6}$ residual WBCs. For example, for an adult transfusion recipient, multiple units of Platelets (4-6 units) are pooled into a single platelet dose. The total (4-6 units) should contain $< 5.0 \times 10^{6}$ residual WBCs. The same recommendation for $< 5.0 \times 10^{6}$ residual WBCs applies if platelet units are pooled before storage, whether filtered before or after storage.

***Or per the device manufacturer's specifications, if different.

B. Equipment/Ancillary Supplies

Equipment must be observed, standardized, and calibrated on a regularly scheduled basis as prescribed in the Standard Operating Procedures Manual (21 CFR 606.60(a)). Such equipment includes, but may not be limited to the ABCS, cell counting instrument(s), scales, blood shaker and STCD.

Supplies and reagents must be used in a manner consistent with instructions provided by the manufacturer (21 CFR 606.65(e)). In addition, all supplies (including filters) and reagents must meet all of the requirements described in 21 CFR 606.65.

C. Operator Training

Operators must have adequate training, education, experience, or combination thereof, to assure competent performance of their assigned functions (21 CFR 606.20(b)). Control

[5] See footnote 1.

over operational variables should be maintained through initial and continued staff training, routine staff participation in leukocyte reduction and periodic assessment of operator performance. We recommend that assessment of operators include scheduled competency assessment and proficiency testing. In addition, we recommend that you develop appropriate training on leukocyte reduction processes and procedures and/or equipment maintenance as updated information becomes available.

D. Standard Operating Procedures

1. Requirements for Standard Operating Procedures (SOPs)

Equipment used for leukocyte reduction must "perform in the manner for which it was designed" (21 CFR 606.60(a)). Supplies and reagents must be used in a manner consistent with the instructions provided by the manufacturer (21 CFR 606.65(e)). Written SOPs must be maintained and must include all steps to be followed in the collection, processing, compatibility testing, storage, and distribution of blood and blood components (21 CFR 606.100(b)). Therefore, you must have written SOPs for each step in the collection and processing of leukocytes reduced Whole Blood and blood components.

2. Additional Requirements and Recommendations to be included in SOPs

- **Sample handling:** Blood samples should be collected, processed, and tested within 48 hours (Ref. 33) (or per the manufacturer's recommendations for the methodology) of leukocyte reduction. The SOP should adequately describe sample storage temperature, time, mixing, etc.

- **Leukocyte reduction filters:** Leukocyte reduction filters are typically component-specific; a filter intended for one component type should not be used with other component types (Ref. 34). Leukocyte reduction filters intended for bedside filtration should not be used for pre-storage leukocyte reduction.

- **Abnormal Red Blood Cell Retention:** Filtration steps should include instructions on how to identify excessive red blood cell retention in the red cell container and tubing above the filter.

- **Re-filtration:** Components that fail to leukocyte reduce appropriately should not be re-filtered, unless re-filtration is specified as appropriate by the device manufacturer in the instructions for use.

- **QC failures:** You must thoroughly investigate any unexplained discrepancy or the failure of a batch to meet any of its specifications (21 CFR 211.192). You should define appropriate criteria for retesting of components, testing of additional components, final labeling, and disposition of components that fail to meet specifications. For example:

- A blood component that requires a filtration time longer than the time interval specified in the manufacturer's instructions for use may be released by the QA unit for transfusion as leukocytes reduced only if testing of that unit confirms acceptable component specifications and there is an investigation of the delayed filtration time.

- Leukocyte reduction by filtration can fail for Whole Blood and Red Blood Cells collected from donors with sickle cell trait (Refs. 3 through 7 and Refs. 35 through 38) or other donor specific factors. Tests for donor-specific factors may reduce the number of components lost to filter failures. To address the investigation of filtration failures, the SOP may include testing the blood component for donor specific factors. The blood unit associated with a filtration failure should not be released for transfusion as leukocytes reduced unless testing of that unit confirms acceptable component specifications.

- **Manufacturer's performance specifications:** You should state the acceptable tolerance specifications for each leukocytes reduced blood component as described by the manufacturer. You should have a procedure addressing the handling of components that do not meet the manufacturer's performance specifications (e.g., use in research or further manufacture).

- **Labeling:** The general requirements for the labeling process are described in 21 CFR 606.120 and must be followed.

V. LABELING

A circular of information must be available for distribution if the product is intended for transfusion (21 CFR 606.122).

Your container labels must comply with 21 CFR 606.121 and 610.60. The container label must include the proper name of the product, in a prominent position, with any appropriate modifier(s) and attributes (21 CFR 606.121(c)(1)). One way to comply with the applicable labeling requirements is through of use of standard terminology. For example, Table 3 lists the proper names of the major leukocytes reduced blood components, followed by the corresponding International Society of Blood Transfusion (ISBT) Code 128 name (Ref. 39). The component labeling should include the phrase "Leukocytes Reduced." The phrases "Leukocytes Removed," "Leukocyte Poor," "Leukocytes Depleted," and other similar terms should not be used in component labeling.

Only those components meeting the recommended residual WBC content for leukocytes reduced blood components or prepared by a validated method known to meet the recommended residual

leukocyte content for leukocytes reduced blood components may be labeled as "Leukocytes Reduced."

Those components (except Whole Blood derived Platelets) that have been tested and found to have a residual WBC count of $< 1.0 \times 10^6$ may be labeled either with the actual residual leukocyte content via a supplemental label or a tie-tag stating: "The residual white blood cell count of this component has been determined to be $< 1.0 \times 10^6$."

Table 3: Component Names for Container Labels

Proper Name	ISBT 128 Name
Whole Blood Leukocytes Reduced	WHOLE BLOOD LEUKOCYTES REDUCED
Red Blood Cells Leukocytes Reduced	RED BLOOD CELLS LEUKOCYTES REDUCED
	APHERESIS RED BLOOD CELLS LEUKOCYTES REDUCED
Platelets Leukocytes Reduced	PLATELETS LEUKOCYTES REDUCED

VI. REPORTING CHANGES TO AN APPROVED BIOLOGICS LICENSE APPLICATION (BLA)

An establishment that distributes leukocytes reduced blood components in interstate commerce must have an approved BLA, including an approval for leukocytes reduced blood components, in accordance with section 351 of the Public Health Service Act.

Licensed establishments must report changes to their approved application(s) in accordance with 21 CFR 601.12. For assistance in reporting your changes see FDA's "Guidance for Industry: Changes to an Approved Application: Biological Products: Human Blood and Blood Components Intended for Transfusion or for Further Manufacture" dated July 2001.[6] The information below is intended to assist you in determining which reporting mechanism is appropriate for a change to your approved BLA, as it applies to the manufacture of leukocytes reduced blood components. You should prominently label each submission with the reporting category under which you are reporting your change, e.g., "Prior Approval Supplement" or "Supplement - Changes Being Effected in 30 Days," or "Annual Report."

A. Prior Approval Supplement (PAS): Changes Requiring Supplement Submission and Approval Prior to Distribution of the Product Made Using the Change (Major Changes) (21 CFR 601.12(b))

Under 21 CFR 601.12(b), changes that have a substantial potential to have an adverse effect on the identity, strength, quality, purity, or potency of the product as they may relate to the safety or effectiveness of the product must be reported to FDA in a Prior Approval Supplement (PAS).

[6] http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm076729.htm.

Under this regulation, the following kinds of manufacturing changes would fall within this category, warranting submission of your request to implement the following changes to your approved BLA as a PAS:

- If you are a licensed blood establishment and you intend to change your Whole Blood manufacturing procedures to include leukocyte reduction.
- If you are currently approved to manufacture leukocytes reduced blood components at a specific facility, and you intend to manufacture leukocytes reduced blood components at a different facility, not under an approved Comparability Protocol.
- If you are approved to manufacture leukocytes reduced blood components, but intend to change your manufacturing process in a manner that presents a substantial potential to have an adverse effect on the identity, strength, quality, purity, or potency of the product as they may relate to the safety or effectiveness of the product and you are not making this change under an approved Comparability Protocol.
- If you intend to manufacture leukocytes reduced blood components using an ABCS device new to your establishment and you are not making this change under an approved Comparability Protocol.
- If you are requesting approval for a Comparability Protocol. The Comparability Protocol described in 21 CFR 601.12(e) is a supplement that describes the specific tests and validation studies and acceptable limits to be achieved to demonstrate the lack of adverse effect for specified types of manufacturing changes on the identity, strength, quality, purity, or potency of the product as they may relate to the safety or effectiveness of the product. A new Comparability Protocol, or a change to an existing one, requires approval from FDA prior to distribution of the product which, if approved, may justify a reduced reporting category for the particular change because the use of the protocol for that type of change reduces the potential risk of an adverse effect (21 CFR 601.12(e)).

A Comparability Protocol is appropriate, but not required, if you wish to implement this change at multiple collection facilities under your direction and control, using the same process to manufacture automated leukocytes reduced components prepared by apheresis.

We consider the recommendations in this guidance document to provide appropriate criteria for a biologics license supplement for leukocytes reduced blood or blood components. You may use an alternate approach if such approach satisfies the requirements of the applicable statutes and regulations. Your alternative procedure(s) may be acceptable if you demonstrate that the resulting leukocytes reduced blood or blood components are equivalent to components manufactured according to the procedures described in this guidance.

You must not distribute in interstate commerce blood components made using a changed manufacturing process requiring a PAS until you have received our approval of your supplement (21 CFR 601.12(b)(3)).

B. Changes Being Effected in 30 Days (CBE-30) Supplement: Changes Requiring Supplement Submission at Least 30 Days Prior to Distribution of the Product Made Using the Change (21 CFR 601.12(c))

Under 21 CFR 601.12(c), changes that have a moderate potential to have an adverse effect on the identity, strength, quality, purity, or potency of the product as they may relate to the safety or effectiveness of the product must be reported to FDA in a Changes Being Effected in 30 days (CBE-30) supplement.

You must submit your request to implement manufacturing changes with a moderate potential for an adverse effect to your approved BLA as a CBE-30 supplement under 21 CFR 601.12(c). The manufacturing changes described below are examples of changes that we believe fall within this category.

- Implementation of a new collection facility for leukocytes reduced Platelets, Pheresis under an approved Comparability Protocol.
- Certain software and hardware upgrades to the leukocyte reduction process for apheresis collection.

You may distribute your blood components made using the change requested in your CBE-30 supplement in interstate commerce 30 days after we receive your supplement, unless we notify you otherwise (21 CFR 601.12(c)(4)).

C. Changes to be Described in an Annual Report (Minor Changes) (21 CFR 601.12(d))

Under 21 CFR 601.12(d), changes in the product, production process, quality controls, equipment, facilities, or responsible personnel that have a minimal potential to have an adverse effect on the identity, strength, quality, purity, or potency of the product as they may relate to the safety or effectiveness of the product must be documented in an annual report submitted each year within 60 days of the anniversary date of approval of the application.

Changes from one type of FDA approved or cleared leukocyte reduction filter to another type of FDA approved or cleared leukocyte reduction filter is an example of a manufacturing change that we believe falls within this category, if implemented according to the instructions in the package insert without modification.

D. Submission of Documents

1. PAS: To comply with the requirements in 21 CFR 601.12(b)(3), the following must be contained in the supplement.

    - Identification of the components involved (i.e., those to be leukocytes reduced) and manufacturing facility(s) or area(s) affected, and a detailed description of the manufacturing change (including device collection technology for leukocyte reduction) (21 CFR 601.12(b)(3)(i) through

(iii)). We recommend that this information be documented in a cover letter and Form FDA 356h.

- Product labeling for each component, if changed (21 CFR 601.12(f)). We recommend submitting a Circular of Information (unless already on file at FDA). To permit assessment of the manufacturing change we recommend including copies of the following SOPs:
  - labeling including acceptable and unacceptable units;
  - a description of training (or an example of training document)
  - component manufacturing;
  - failure investigation;
  - quality control including sampling scheme, sample handlings, tracking and trending; and
  - quarantine and disposition of unsuitable products.
- A reference list of relevant SOPs (21 CFR 601.12(b)(3)(vii)). We recommend submitting procedures unless they have been previously approved by the FDA.
- Relevant validation protocols and data (21 CFR 601.12(b)(3)(vi)). We recommend a summary of the validation protocol, including failure investigations.
- A description of the methods used and studies performed to evaluate the effect of the change and the data derived from such studies (21 CFR 601.12(b)(3)(iv) through (v)). We recommend submitting the following information and data:
  - the device manufacturer;
  - the device type;
  - blood unit number;
  - component description;
  - date of collection;
  - date of testing;
  - result interpretation(s);
  - the identity of the person performing the testing;
  - the identity of the collection facility;
  - the identity of the testing facility;
  - evidence of QA oversight; and
  - expected component specifications for the residual WBC count.

Additionally, we recommend two months of QC data for component volume and residual WBC count per component.

2. Comparability Protocol (21 CFR 601.12(e)). If you are an establishment with multiple manufacturing sites and wish to submit a Comparability Protocol to justify a reduced reporting category for a manufacturing change at multiple sites (see section VI.D.4 below), you must submit that protocol as a PAS (21 CFR 601.12(e)). In addition to the information listed in section VI.D.1 above, we recommend that you include the following:

- Implementation plan; and
- Proposed reporting category for changes made under the proposed Comparability Protocol.

3. CBE-30 submissions (excluding new facilities under an approved Comparability Protocol). Under 21 CFR 601.12(c)(3) and 21 CFR 601.12(b)(3)(i) through (vii), the following information must be contained in your CBE-30 submission:

   - Identification of the components involved (i.e., those to be leukocytes reduced) and manufacturing site(s) or area(s) affected, and a detailed description of the proposed manufacturing change (including device collection technology for leukocyte reduction). We recommend that you document this information in a cover letter and Form FDA 356h. To permit assessment of the documented manufacturing change, we recommend that you include copies of any new or revised SOPs.
   - Product labeling for each component (21 CFR 601.12(f)).
   - Relevant validation protocols and data. We recommend that you submit a copy of the validation summary, including the investigation of any failures.
   - The data derived from studies. We recommend two months of QC data for component volume, residual WBC count per component and percent recovery or minimum Red Blood Cell volume if applicable.
   - A description of the methods used and studies performed to evaluate the effect of the change and the data derived from such studies. We recommend that you submit the following information:
     - evidence of quality control oversight, including review and approval of manufacturing records by a quality assurance unit; and
     - expected component specifications including residual WBC count and percent component recovery, if applicable.

4. CBE-30 submissions for new facilities under an approved Comparability Protocol. To comply with 21 CFR 601.12(c)(3) and 21 CFR 601.12(b)(3)(i) through (vii), the following information must be included:

   - Identification of the components involved (i.e., those blood component(s) to be leukocytes reduced) and new manufacturing site(s) or area(s) affected, and a description of the implementation plan (manufacturing change including device collection technology for leukocyte reduction). Additionally, we recommend that this information be documented in a cover letter and FDA Form 356h.

- Relevant validation protocols and data. We recommend a summary of the validation protocol, including failure investigations to meet the requirement.
- The data derived from studies. We recommend two months of QC data.

In addition, you should include the submission tracking number (STN) of the approved Comparability Protocol, and the STN(s) of changes to the SOPs associated with an approved Comparability Protocol.

5. Annual Report. To comply with 21 CFR 601.12(d)(3), the following information must be included:

   - a list of all products involved; and
   - a full description of the manufacturing and controls changes including the manufacturing site(s) or area(a) involved.

VII. CONTACT INFORMATION

Questions about licensing and specific review questions about leukocyte reduction should be directed to the Office of Blood Research and Review, Division of Blood Applications (DBA). Submit all registration forms (Form FDA 2830) and licensure applications/supplements to the Director, Center for Biologics Evaluation and Research (CBER). Table 4 presents FDA contact information regarding leukocyte reduction.

Table 4: FDA Contact Information

Submissions: Registrations License Applications	Director, Center for Biologics Evaluation and Research, HFM-370, Food and Drug Administration, c/o Document Control Center, HFM-99, 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448.
Review Questions: Licensing Red Blood Cells Whole Blood Platelets Platelets, Pheresis Plasma	Director, Division of Blood Applications, HFM-370, Food and Drug Administration, c/o Document Control Center, HFM-99, 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, Voice 301-827-3543; Fax 301-827-3534.

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APPENDIX

QC testing and sample size calculations based on the hypergeometric distribution

Section III.F of this document describes a two-stage sampling approach to process validation based on the binomial distribution. As discussed in Section IV.A, we consider this approach to be a scientifically sound statistical sampling and analytic plan for QC testing as well as for process validation. One limitation of the binomial distribution-based approach, however, is that it assumes that the population of components being sampled from is infinite. This assumption may not be ideal for sampling performed for monthly QC testing.

A two-stage sampling approach based on the hypergeometric distribution may be used for monthly QC. Unlike the binomial approach, the hypergeometric approach assumes that a finite number of components will be manufactured during a monthly QC period. In general, the sample size for QC testing under the hypergeometric approach will be smaller than that for binomial distribution-based sampling. However, you may prefer to use binomial distribution-based sampling for other reasons, including familiarity and simplicity. The decrease in sample size for the hypergeometric approach relative to the binomial approach is most noticeable when the total number of collections to be performed during a given QC period is small.

The two-stage hypergeometric approach is similar in concept to the two-stage binomial approach described in Section III.F. In the first stage, an initial representative sample of components with a predetermined sample size should be tested consecutively. Identified non-process failures may be excluded from the sample size and replaced. Conformance can be demonstrated if an acceptable number of process failures are observed in this sample. In the event that there is one more process failure than permitted in the initial sample, a second sample of components may be tested consecutively. Conformance can be demonstrated if no further process failures are observed in the second sample. The size of the second sample will depend on the size of the first sample, and may encompass the entire remaining population of components to be tested during a given QC period.

Table A details possible sampling plans and sample sizes for two-stage QC testing based on the hypergeometric distribution for components other than platelets. Table B provides sampling plans and sample sizes for platelet yield QC testing. Each of the sampling plans contained in the tables satisfies the statistical criteria for successful QC testing provided in Section IV.A. The tables may be used as follows:

1. Determine the number of components that will be manufactured during a given QC period (i.e., one month of a given process at a given site). This corresponds to the population size contained in the first column of the table. Choose the appropriate row of the table corresponding to the population size for the given QC period. In general, this will not be known precisely in advance and will need to be estimated. It is important that the estimated population size be at least as large as the true population size. That is, you should choose the maximum possible number of components for a given QC period rather than, e.g., an average number of components.

2. Once you have identified the appropriate row corresponding to the population size for a given QC period, you can choose from up to three possible two-stage sampling plans, distinguished by the number of failures allowed in the first stage: zero failures, one failure or two failures.
3. The two columns headed by the sampling plan chosen in Step 2 above contain the required sample sizes for the first and second stages of the sampling, respectively. In some cases the sample size for the second stage is denoted "All." This indicates that every component in the given QC period must be tested in the event of one excess process failure in the first sampling stage. In other cases, the sample size for the second stage is denoted with a dash. This indicates that a two-stage approach is not possible for the given population size and first stage sample, and that one excess process failure in the first stage means overall failure of the QC testing for that QC period.

As an example of this approach, suppose that you intend to collect 100 leukocyte-reduced plasma components on a given instrument at a given site during the next month. Note first that you may test every one of these 100 units and, if 4 or fewer process failures are observed, the process failure rate is less than 5% for that QC period. Adopting instead a statistical sampling strategy, you may consult Table A and choose one of the three sampling plans provided in the row corresponding to population size of 100. Suppose you choose the "zero failures allowed" sampling plan in this row, which specifies a first stage sample size of 45 components and a second stage sample size of 34 components. You would therefore test the first 45 consecutive components for that month. If no process failures were observed among these 45 components, the process would be considered to be in control and no further QC testing would be required during that month. If one process failure is observed among the first 45 components, you would test an additional 34 consecutive components. If no further process failures were observed among the second stage sample of 34 components, the process would be considered to be in control and no further testing would be required. If two or more process failures were observed during the first stage sample of 45 components or if one or more process failures were observed during the second stage sample of 34, the process would not be in control and QC testing would have failed.

Statistical derivation of the sample sizes in Tables A and B

In Table A, for each fixed population size, N, and number of allowed failures, m, the first stage sample size was calculated as the minimum number n_1 such the probability of observing m or fewer process failures in a sample of size n_1 from a population of size N is at most 5% when the true process failure rate is 5% or more. For example, assume that 100 components will be collected during a QC period. Using the "zero failures allowed" first stage sampling plan, it can be calculated based on the hypergeometric probability distribution that the probability of observing zero failures in a sample of 45 components is 0.0462 if the true failure rate is at least 5% (that is, if there are at least 5 process failures among the 100 components to be collected). On the other hand, the probability of observing zero failures in a sample of 44 components is 0.0507. Therefore, 45 is the minimum sample size such that the probability of observing zero failures is less than 5% when the true failure rate of at least 5%.

Once the first stage sample size has been determined, to obtain the second stage sample size, we first calculated 0.05 minus the probability of observing *m* or fewer process failures in the first stage when the true failure rate is at least 5%. Continuing our example above, this quantity would be 0.05 – 0.0462 = 0.0038. The second stage sample size was then calculated as the minimum number n_2 such that the probability of observing (i) one failure among the first n_1 samples at stage one and (ii) no failures among the n_2 samples at stage two is less than 0.05 minus the probability of observing *m* or fewer process failures in the first stage when the true failure rate is at least 5%. In the example here, with $n_2 = 33$, this probability is 0.0044, which is not less than 0.0038, whereas with $n_2 = 34$, this probability is 0.0036 which is less than 0.0038. Therefore, the required sample size at stage two for this example is 34. Romeu (Ref. 41) adopts the same algorithm for an infinite population size based on the binomial distribution.

The derivation of the sample sizes in Table B followed the same process, but was based on ruling out a true failure rate of at least 25% rather than 5% as in Table A. Note that hypergeometric sampling schemes other than those presented in Tables A and B are possible. For instance, in some cases it is possible to use a larger first stage sample size and smaller second stage than that provided in the tables. We will consider any sampling plan that meets the criteria described in Section IV.A.

Table A. Sampling scheme for quality control testing based on hypergeometric distribution (95%/95%).

Use for all QA and monitoring assessments under Section IV, except in accessing platelet yield. See Table B for assessment of platelet yield.

Population size[2]	95%/95%[1] 95% confidence that more than 95% of the components meet the standard						
	# of failures allowed in population	Sample size					
		0 failures allowed	Add[3]	1 failure allowed	Add[3]	2 failures allowed	Add[3]
30	1	23	All[4]	30	-	-	-
31	1	24	All	31	-	-	-
32	1	25	All	32	-	-	-
33	1	26	All	33	-	-	-
34	1	26	All	34	-	-	-
35	1	27	All	35	-	-	-
36	1	28	All	36	-	-	-
37	1	29	All	37	-	-	-
38	1	30	All	38	-	-	-
39	1	30	All	39	-	-	-
40	1	31	All	39	-	-	-
45	2	28	15[5]	39	All[6]	45	-
50	2	31	18	43	All	50	-
55	2	35	17	48	All	55	-
60	2	38	19	52	All	60	-
65	3	34	23	49	13	59	All[7]
70	3	37	23	52	17	63	All
75	3	39	29	56	17	68	All
80	3	42	28	60	17	72	All
85	4	38	30	56	19	69	13
90	4	40	34	59	22	73	14
95	4	42	41	62	25	77	15
100	4	45	34	65	30	81	16
120	5	47	40	69	37	87	25
140	6	48	51	72	44	92	28
160	7	49	62	75	39	95	42
180	8	50	64	77	41	98	44
200	9	51	61	78	51	101	39
220	10	52	57	79	62	103	42
240	11	52	73	80	71	104	55
260	12	53	61	81	69	106	47
280	13	53	72	82	63	107	52
300	14	54	60	83	58	108	56
320	15	54	67	83	94	109	57
340	16	54	75	84	65	110	55
360	17	54	91	85	57	111	53

Population size[2]	95%/95%[1] 95% confidence that more than 95% of the components meet the standard						
	# of failures allowed in population	Sample size					
		0 failures allowed	Add[3]	1 failure allowed	Add[3]	2 failures allowed	Add[3]
380	18	55	65	85	66	111	70
400	19	55	70	85	85	112	60
450	22	54	78	84	111	111	62
500	24	56	69	87	70	114	76
550	27	55	76	86	72	113	75
600	29	56	85	88	74	116	67
650	32	56	69	87	78	115	66
700	34	57	71	89	70	117	73
750	37	56	80	88	74	116	73
800	39	57	78	89	95	118	71
850	42	56	96	89	67	117	72
900	44	57	86	90	73	119	66
950	47	57	73	89	81	118	67
1000	49	57	95	90	86	119	82
1500	74	58	81	91	95	121	75
2000	99	58	92	92	78	122	72
2500	124	58	101	92	91	122	91
3000	149	58	111	92	109	123	71
3500	174	58	124	93	74	123	77
4000	199	58	149	93	77	123	83
4500	224	59	79	93	79	123	89
5000	249	59	80	93	81	123	95
6000	299	59	81	93	85	123	113
7000	349	59	82	93	88	124	72
8000	399	59	83	93	91	124	74
9000	449	59	84	93	93	124	76
10000	499	59	85	93	95	124	77
11000	549	59	85	93	97	124	79
12000	599	59	85	93	99	124	80
13000	649	59	86	93	100	124	81
14000	699	59	86	93	102	124	82
15000	749	59	86	93	103	124	83
20000	999	59	87	93	108	124	86
25000	1249	59	88	93	112	124	88
30000	1499	59	88	93	116	124	90
35000	1749	59	89	93	119	124	91
40000	1999	59	89	93	121	124	92
45000	2249	59	89	93	123	124	93
50000	2499	59	89	93	125	124	93
55000	2749	59	89	93	126	124	94

Population size[2]	# of failures allowed in population	95%/95%[1] 95% confidence that more than 95% of the components meet the standard — Sample size: 0 failures allowed	Add[3]	1 failure allowed	Add[3]	2 failures allowed	Add[3]
60000	2999	59	89	93	128	124	94
2.00E+07[8]	999999	59	90	93	163	124	100

[1]The inference that with 95% confidence more than 95% of the components meet the standard is only applicable to the finite population from which the samples are drawn.

[2]The population size is the total number of products manufactured in a given quality control (QC) period (e.g., one month for a monthly QC) at a given site using a given instrument, etc.

[3]Additional testing if allowable process failures are exceeded (see footnotes 4 through 7)

[4]For a population size of 30, if you select a sample size of 23 and find one failure, test all the remaining components and no additional failures are allowed.

[5]For a population size of 45, if you select a sample size of 28 and find one failure, test 15 additional samples and no additional failures are allowed.

[6]For a population size of 45, if you select a sample size of 39 and find two failures, test the remaining components and no additional failures are allowed.

[7]For a population size of 65, if you select a sample size of 59 and find three failures, test the remaining components and no additional failures are allowed.

[8]With a very large population size, the sample size based on hypergeometric distribution converges to the sample size based on the binomial distribution although the convergence is very slow in some cases.

Table B. Sampling scheme for quality control testing based on hypergeometric distribution (95%/75%).

Under 21 CFR 640.24(c), the platelet product must yield an acceptable count in at least 75 percent of the units tested. Table B applies only to platelet yield and does not pertain to the other aspects of QC testing of platelet products (e.g., assessing white count, pH) where the appropriate measure is 95%/95%.

Population size[2]	95%/75%[1] 95% confidence that more than 75% of the components meet the standard						
	# of failures allowed in population	Sample size					
		0 failures allowed	Add[3]	1 failure allowed	Add[3]	2 failures allowed	Add[3]
30	7	9	6	13	8	17	6
31	7	9	7	14	5	18	5
32	7	9	9	14	8	18	9
33	8	9	6	13	8	17	7
34	8	9	7	14	5	18	5
35	8	9	8	14	7	18	8
36	8	9	14	15	5	19	6
37	9	9	7	14	5	18	5
38	9	9	8	14	7	18	8
39	9	9	10	15	5	19	5
40	9	10	7	15	6	19	9
45	11	9	9	14	8	19	5
50	12	9	15	15	6	19	10
55	13	10	8	15	10	20	7
60	14	10	9	16	7	21	6
65	16	10	7	15	9	20	7
70	17	10	8	16	6	20	14
75	18	10	10	16	8	21	7
80	19	10	11	16	9	21	10
85	21	10	9	16	7	21	7
90	22	10	10	16	8	21	8
95	23	10	11	16	10	21	13
100	24	10	14	16	14	22	7
120	29	10	17	17	7	22	9
140	34	11	8	17	8	22	10
160	39	11	8	17	9	22	13
180	44	11	9	17	9	22	17
200	49	11	9	17	9	23	7
220	54	11	9	17	10	23	8
240	59	11	9	17	10	23	8
260	64	11	9	17	10	23	8
280	69	11	9	17	11	23	8
300	74	11	9	17	11	23	9
320	79	11	10	17	11	23	9

Population size[2]	95%/75%[1] 95% confidence that more than 75% of the components meet the standard						
	# of failures allowed in population	Sample size					
		0 failures allowed	Add[3]	1 failure allowed	Add[3]	2 failures allowed	Add[3]
340	84	11	10	17	12	23	9
360	89	11	10	17	12	23	9
380	94	11	10	17	12	23	9
400	99	11	10	17	12	23	10
450	112	11	10	17	12	23	9
500	124	11	10	17	13	23	10
550	137	11	10	17	12	23	10
600	149	11	10	17	14	23	11
650	162	11	10	17	13	23	10
700	174	11	10	17	14	23	11
750	187	11	10	17	14	23	11
800	199	11	10	17	15	23	12
850	212	11	10	17	14	23	11
900	224	11	10	17	15	23	12
950	237	11	10	17	15	23	12
1000	249	11	10	17	16	23	12
1500	374	11	11	17	17	23	13
2000	499	11	11	17	19	23	14
2500	624	11	11	17	20	23	14
3000	749	11	11	17	21	23	15
3500	874	11	11	17	21	23	15
4000	999	11	11	17	22	23	15
4500	1124	11	11	17	23	23	15
5000	1249	11	11	17	23	23	15
6000	1499	11	11	17	24	23	16
7000	1749	11	11	17	26	23	16
8000	1999	11	11	17	27	23	16
9000	2249	11	11	17	28	23	16
10000	2499	11	11	17	30	23	16
11000	2749	11	11	17	33	23	16
12000	2999	11	11	17	43	23	16
13000	3249	11	11	18	8	23	16
14000	3499	11	11	18	8	23	16
15000	3749	11	11	18	8	23	16
20000	4999	11	11	18	8	23	16
25000	6249	11	11	18	8	23	17
30000	7499	11	11	18	8	23	17
35000	8749	11	11	18	8	23	17
40000	9999	11	11	18	8	23	17
45000	11249	11	11	18	8	23	17

Population size[2]	95%/75%[1] 95% confidence that more than 75% of the components meet the standard						
	# of failures allowed in population	Sample size					
		0 failures allowed	Add[3]	1 failure allowed	Add[3]	2 failures allowed	Add[3]
50000	12499	11	11	18	8	23	17
55000	13749	11	11	18	8	23	17
60000	14999	11	11	18	8	23	17
2.00E+07[8]	4999999	11	11	18	8	23	17

[1]The inference that with 95% confidence more than 75% of the components meet the standard is only applicable to the finite population from which the samples are drawn.
[2]The population size is the total number of products manufactured in a given quality control (QC) period (e.g., one month for a monthly QC) at a given site using a given instrument, etc.
[3]Additional testing if allowable process failures are exceeded (see footnotes 4 through 7)
[4]For a population size of 30, if you select a sample size of 23 and find one failure, test all the remaining components and no additional failures are allowed.
[5]For a population size of 45, if you select a sample size of 28 and find one failure, test 15 additional samples and no additional failures are allowed.
[6]For a population size of 45, if you select a sample size of 39 and find two failures, test the remaining components and no additional failures are allowed.
[7]For a population size of 65, if you select a sample size of 59 and find three failures, test the remaining components and no additional failures are allowed.
[8]With a very large population size, the sample size based on hypergeometric distribution converges to the sample size based on the binomial distribution although the convergence is very slow in some cases.