Clinical trial and local process evaluation of an apheresis system for preparation of white cell-reduced platelet components

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BACKGROUND: A new method for the consistent preparation of white cell (WBC)-reduced plateletpheresis components, the Spectra Leukoreduction System (LRS), was evaluated by clinical trial and local process validation. The centrifuge-based system was projected to decrease the WBC content of plateletpheresis components to a level below 1×10^6 per unit. Phase I and II clinical trials were performed. The manufacturer's claims were then tested at the local level with an ongoing quality assurance program.

STUDY DESIGN AND METHODS: In Phase I, a crossover analysis of five subjects compared LRS to standard plateletpheresis procedures in collection efficiency and component quality: a panel of in vitro measures was taken on Day 0 and Day 5. In Phase II, the LRS process was tested on a larger scale (n = 57; control = 58) with component transfusion. Finally, validation, determination of degree of conformance with standards, and ongoing quality control were performed locally on a newly installed instrument.

RESULTS: Phase I and II trials revealed no significant differences between LRS and control units in donor or recipient safety and comfort, platelet function and yield, or component volume. WBC per-unit values were significantly different: the LRS median per unit was 3.2×10^4 WBCs, versus 81.4×10^4 for control units. Assessment of process capability gave an estimate of 99-percent confidence that 99.5 percent of LRS units would be WBC reduced to $<1 \times 10^6$ WBCs. Local process validation and quality control revealed 90-percent confidence that 99 percent of the units would be WBC reduced and 99.9-percent confidence that 75 percent would exceed platelet yield standards; the process was stable over time.

CONCLUSIONS: The LRS is safe for apheresis and the component produced is safe for transfusion with platelet function and yield equivalent to controls and WBC reduction superior to controls. Local process evaluation confirmed that component quality meets the goals of the institution.

Cellular blood components contain 10^9 to 10^{10} white cells (WBCs) that are responsible for the majority of transfusion-related complications. Reduction in WBC levels has been shown to prevent or delay complications such as febrile nonhemolytic transfusion reactions and HLA alloimmunization.[1-3] Recent data from the multi-institution clinical Trial to Reduce Alloimmunization to Platelets (TRAP) show that refractoriness to platelet transfusion decreased from 13 to 4 percent with the use of WBC-reduced platelets.[4] Current data also suggest that WBC reduction in platelet components within 1 to 2 days of production decreases the concentration of plasma cytokines, which reduces the incidence of recurrent FNHTRs to levels equal those in WBC-reduced red cell components.[5,6] Further benefits of WBC reduction may include a decrease in expected cytomegalovirus transmission rates, prevention of reactivation of viral infections in recipients, and reduced transfusion-mediated immunomodulation.[7-12]

MATERIALS AND METHODS

Development of a centrifuge-based WBC-reduction chamber and apheresis system

A centrifuge-based WBC-reduction chamber has been developed (Spectra Leukoreduction System [LRS], COBE BCT, Lakewood, CO) that is projected to decrease the WBC content of plateletpheresis components to a level below 1×10^6 per unit at least 99 percent of the time.[13] The chamber consists of two plastic cones made from a polyester copolymer (copolyester) ultrasonically joined at their greatest diameters to form a single, empty chamber (11-mL maximum volume) that is placed into the collection line of the rotat-

ABBREVIATIONS: LRS = (Spectra) Leukoreduction System; WBC(s) = white cell(s).

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ing blood collection set (Fig. 1). WBC reduction occurs simultaneously with platelet collection; there is no conventional fiber filter in this system. Rather, filtration is similar to fixed particle-bed filtration, where, instead of particulate matter such as sand, the platelets themselves are the particle bed medium. A saturated, "fluidized," platelet particle bed is created by pulling a solution of platelets and plasma into and through the rotating conical chamber by means of a pump. As flow begins, the plasma flow velocities through the chamber are directed radially inward and are not sufficient to overcome the platelet sedimentation velocities directed radially outward (opposite the plasma flow). As more platelets enter the chamber, they continue to accumulate to the point at which the resident platelet bed becomes "saturated." At saturation, the interstitial plasma velocity between platelet particles is only slightly greater than the plasma sedimentation velocity, and the flow past any single platelet in the bed is just large enough to lift the platelet slowly out of the gravitational field. The WBCs that enter with the platelets sediment much faster than platelets and remain below (radially outward from) the platelets; the saturated platelet bed is virtually impervious to WBCs. This WBC-reduction method is called fluidized particle-bed separation.

Development of the LRS has involved minimal modification to the existing system (Spectra Version 4, COBE). The new chamber is fabricated of copolyester plastic that is already used elsewhere in the commercially available platelet collection set. The chamber is connected to a Spectra platelet collection blood set at the factory. Except for the addition of this chamber to the collect line, the portion of the centrifuge that holds the separation channel during operation, the configuration of the set remains unchanged. The conical shape is designed so that the chamber can accommodate a range of collection flow rates from 2 mL per minute to 8 mL per minute. Platelets remaining in the LRS chamber are recovered during platelet inventory recovery, while contaminating WBCs are left behind. Continuous, real-time monitoring of apheresis stability is also provided by the LRS collect concentration monitor, an optical device in the collection line that measures the light scatter and optical density characteristics of collected platelet components. If a component may contain more than a predetermined WBC concentration, an alarm notifies the operator that component cellular contents should be verified before issue.

Fig. 1. The LRS chamber during platelet apheresis. The LRS chamber is shown mid-procedure, within the spinning centrifuge. It is positioned within the collection line of the blood tubing collection channel. First-stage platelet-rich plasma separation from whole blood is shown at right, above the time stamp. The LRS chamber is saturated with platelets, which creates a fluidized particle bed for separation of residual WBCs. The step gradations of the chamber work to counteract the Coriolis force, thereby increasing the effectiveness of WBC retention in the chamber.

Clinical trial

The clinical trial was divided into two phases: the Phase I trial focused on component quality as measured by platelet function in vitro, component WBC level, and platelet yield. The Phase II trial focused on the overall performance of the LRS; the study was expanded to a larger donor population and the component was transfused. Component WBC content, platelet yield, operator interface, software performance, and donor comfort were evaluated. The sample size was chosen to confirm the goal of detecting a WBC-reduction failure rate in the LRS units of $\leq$1 percent with $\geq$95-percent confidence.

After the clinical trial, an LRS instrument was installed at the local institution. Process evaluation was performed by using the guidelines proposed by the Biomedical Excellence for Safer Transfusion (BEST) working group: 1) validation was performed to verify the manufacturer's claims and establish the distribution of values for the local population; 2) the stability of the process over time was monitored with Levey-Jennings control charts; and 3) conformance with standards was assessed.[14] This form of analysis allows local determination of the degree of confidence with which the component meets standards and of whether it does so consistently over time. On the basis of the potential consequences of a Type 2 error, the blood center medical staff determined as acceptable the goal of detecting $\leq$5-percent WBC failure with $\geq$90-percent confidence.

Subjects

The Phase I and II trials were performed under nonsignificant-risk investigational-device exemptions, with each phase separately approved by the Investigational Review Board of the University of California, San Francisco. Donors were screened according to the standard criteria for blood donation at the Blood Centers of the Pacific, Irwin Center. Signed

consents were obtained from all subjects placed on the LRS during both Phase I and II trials.

Study design: Phase I

Procedure. The donors (n = 5) underwent apheresis twice in random sequence, once on the current (Version 4) Spectra system (controls) and once on the LRS. The routine plateletpheresis procedure was followed for the LRS and control procedures with the exception that both control and test procedures were 100 minutes in duration. The LRS donations were monitored by a computer-based data-management system (APOLLOnet, COBE BCT) as well as by the use of research and routine forms that were completed for each procedure. APOLLOnet can be attached to the Spectra system for monitoring and logging equipment values (e.g., flow rates, alarm histories, pressures) during the procedure.

Donor values. Preplateletpheresis and postplateletpheresis counts were performed for all procedures.

Component analysis. WBC concentration was measured at COBE BCT by Nageotte hemocytometer, and flow cytometry was performed by a published method developed in that laboratory.[15] WBC values determined at COBE BCT were confirmed by parallel Nageotte hemocytometer counts performed on 30 units at the Blood Centers of the Pacific. For the component of each procedure, the platelet count per unit volume was measured with a hematology analyzer (9110, Baker, Allentown, PA), product volume was determined gravimetrically, and the component platelet yield was calculated. In vitro platelet function was studied on Days 0 and 5. Measurements included pH at room temperature, C3a and C5b-9 complex complement studies, platelet morphology studies,[16] a platelet shape change assay utilizing ADP,[17,18] platelet activation studies (P-selectin),[19] hypotonic shock response,[20] and platelet aggregation analysis (ADP 10 μ*M* and epinephrine 10 μ*M*, and collagen 5 μg/mL and epinephrine 10 μ*M*).[21] The platelet components collected during the Phase I study were not used for transfusion.

Study design: Phase II

Procedure. Donors were randomly assigned to undergo apheresis on either the LRS (n = 57) or the Spectra Version 4 instrument (n = 58). LRS procedures were performed on one instrument by eight collection operators. The control apheresis procedures were performed at the same site according to routine procedure at the Blood Centers of the Pacific. The LRS procedure was monitored by APOLLOnet as well as by the use of research and routine forms.

Donor values. Predonation and postdonation WBC and platelet counts were performed on all subjects. Donor comfort was assessed by the nurses, and any discomfort was noted on the forms.

Recipient values. Hospital transfusion service logs were reviewed at the end of the study for reports of transfusion reactions associated with LRS or control units. Platelet corrected count increment values were not collected because of inconsistent posttransfusion platelet count determinations and the variability of recipient clinical status.

Component values. Component WBC count, platelet yield, and volume were measured as described for Phase I. Fourteen single-needle procedures were compared to 44 double-needle procedures.

Statistical analysis of clinical trials

Paired *t* tests were used for within-subject data analysis (Phase I), and unpaired *t* tests were used for between-group data analysis (Phase II). Both residual WBC count and platelet yield values were evaluated by using full enumeration methods.[14] The distribution of platelet yield and log-transformed WBC counts were evaluated with cumulative frequency plots of ranked values.

Method of process evaluation

The residual WBC counts and the platelet counts for the LRS units were evaluated as recommended by Dumont et al.[14] to include 1) process validation, 2) stability of the process over time, and 3) capability assessment and conformance with standards. Process validation was performed with a normal probability plot. This form of data display reveals the normality of the distribution of data (the regression is linear for data that are normally distributed), allowing probabilistic inferences about mean and SD. It also reveals the degree to which the process is accurate and precise within specification. Each data point was assigned a cumulative frequency, which was plotted on a normal probability plot. The linearity of the distribution and the percentage of data points less than the previously specified limit (standard cutoff value) were inferred directly from the plot. Process control and stability were evaluated with the use of a Levey-Jennings control chart. The mean and SD of $\log_{10}$ WBC count and platelet yield (n = 4) were plotted over time. The samples for ongoing process control were obtained from the validated LRS instrument, operated by staffers who had participated in the validation study.

Capability assessment and conformance with standards were evaluated with the use of consecutive components accumulated during the validation and/or process stability evaluations. The following equation forms the basis for the analysis:

$$\text{Mean} - (k_7 \times \text{SD}) < \text{standard cutoff value.} \quad (1)$$

The mean and SD describes an estimate of the distribution of the population. The k_7 tolerance factor, obtained from a k_7 table, allows adjustment of the estimate to the degree that the true population mean and SD are known, which are functions of the sample size.[22] The table charts the value of the k_7 factor for increasing sample size given certain confidence and tolerance values. The degree of confidence that

a log-normal distribution of WBC counts has a certain percentage of the units less than the standard cutoff value can be easily determined with a k_7 table by the technique of Dumont et al.[14] The observed mean $\log_{10}$ WBC value is compared to the "maximum acceptable mean $\log_{10}$ WBC value," calculated by using the standard cutoff value of 1×10^6 WBCs per unit, the observed SD of the population, and a k_7 factor chosen for sample size and confidence goals, as follows:

$$\text{Maximum acceptable mean } \log_{10} \text{WBC value} = \log_{10} \text{standard cutoff value} - (k_7 \times \log_{10} \text{SD}). \quad (2)$$

The observed mean $\log_{10}$ WBC value must be less than the maximum acceptable mean $\log_{10}$ WBC value to ensure the chosen degree of confidence and passage rate. For platelet yield analysis, in which standards require $>3 \times 10^{11}$ platelets per unit, a minimum acceptable mean platelet yield value is determined as follows:

$$\text{Minimum acceptable mean} = 3 \times 10^{11} + (k_7 \times \text{SD}). \quad (3)$$

The observed mean platelet yield must be greater than the minimum acceptable mean to ensure a chosen degree of confidence and passage rate. The confidence (%) in meeting standard cutoff values and failure values obtained locally can also be evaluated against the manufacturer's claim.

RESULTS

Phase I clinical trial

The Phase I trial studies showed the LRS unit to be essentially identical in vitro to the control unit (Table 1). Both groups of platelets were effectively maintained in vitro over the 5-day storage period. There was a difference ($p<0.03$) in platelet collection efficiency in the LRS and the control procedures, with the mean LRS platelet concentration higher than that of controls (1745 ± 110 and $1616 \pm 123 \times 10^9$/L, respectively); there was no significant difference in the final platelet yield. Platelet concentrations increased during storage for both control (+13%) and LRS (+7%) units, most likely because of the disassociation of small platelet aggregates in the Day 0 component after storage on the flatbed agitator.[23] The Day 5 platelet aggregation response to collagen and epinephrine was slightly stronger in LRS units than in controls ($98 \pm 30\%$ and $73 \pm 27\%$, respectively; $p<0.02$). Lactate dehydrogenase in the Day 0 LRS units was significantly lower than that in controls ($p<0.002$). These statistical differences were interpreted as clinically insignificant.

Four of five LRS units were identified by the instrument's collect concentration monitor as containing $<1 \times 10^6$ WBCs. The highest measured WBC count in these four units (1.5×10^5 WBCs) was less than the lowest value in the control units (4.4×10^5 WBCs) ($p<0.003$). The one LRS unit flagged by the instrument as potentially having a WBC count $>1 \times 10^6$ was measured to have 5.3×10^7 WBCs. This flagged unit, suspected a priori to contain $>1 \times 10^6$ WBCs, was not included in the WBC analysis of LRS units.

Phase II clinical trial

There was no statistical difference between preapheresis and postapheresis WBC counts of the LRS subjects ($p<0.32$) or in the preapheresis WBC counts of the LRS and control subjects ($p<0.06$). There was a statistical difference at the 0.04 level between preapheresis platelet counts of the LRS

TABLE 1. Summary of Phase I component results (n = 5 subjects)

	Day 0			Day 5		
	Control units (Version 4)	LRS units	p values	Control units (Version 4)	LRS units	p values
pH (room temperature)	7.27 ± 0.15	7.25 ± 0.12	0.50	7.18 ± 0.28	7.25 ± 0.06	0.60
pCO_2 (mmHg)	57.3 ± 21.1	58.9 ± 25.4	0.77	30.5 ± 3.2	29.9 ± 4.0	0.63
pO_2 (mmHg)	125 ± 27	127 ± 28	0.81	62 ± 18	58 ± 14	0.75
Morphology						
(shape change score, 400 max)	365 ± 21	359 ± 17	0.61	354 ± 17	352 ± 13	0.85
Percentage of discs	77.6 ± 12.1	78.2 ± 5.1	0.91	67.4 ± 13.0	63.8 ± 11.2	0.57
Hypotonic shock response (% recovery)	36.4 ± 10.7	39.8 ± 11.1	0.06	40.4 ± 8.8	42.8 ± 4.7	0.47
Aggregation (%)						
Epinephrine + ADP	76.6 ± 21.7	88.2 ± 30.9	0.36	42.4 ± 34.3	45.4 ± 42.6	0.89
Epinephrine + Collagen	107 ± 14	113 ± 11	0.29	73 ± 27	98 ± 30	0.02
Lactate (mmol/L)	1.11 ± 0.13	0.9 ± 0.29	0.11	6.91 ± 3.42	6.11 ± 0.08	0.67
Lactate dehydrogenase (U/L)	93.5 ± 14.3	77.5 ± 11.1	0.002	95.5 ± 11.8	89.1 ± 13.6	0.22
P-selectin (%)	17.6 ± 10	17.3 ± 16	0.94	37.2 ± 13	35.6 ± 22	0.84
C3a (ng/mL)	592 ± 188	533 ± 217	0.15	2057 ± 248	2070 ± 249	0.93
C5b9 (ng/mL)	65.8 ± 19.1	44.8 ± 29	0.12	214 ± 46	184 ± 79	0.23
Platelet concentration ($\times 10^9$/L)	1616 ± 123	1745 ± 110	0.03	1828 ± 135	1868 ± 258	0.75
MPV (FL)	7.5 ± 0.5	7.5 ± 0.4	1	6.9 ± 0.3	6.9 ± 0.5	0.85
Platelet yield ($\times 10^{11}$/unit)	5.92 ± 1.65	6.08 ± 2.10	0.56			
Residual WBCs						
Median WBCs ($\times 10^6$/unit)	6.17	0.05	0.003*			

and control subjects; further analysis revealed that donor preapheresis platelet count (cutoff value, 300 x 10^9/L) had no significant influence on component platelet yield (p<0.46 LRS units, p<0.22 control units) or on residualWBC count (p<0.18 LRS units, p<0.21 control units).

The nurses were comfortable with the LRS instrument, and, once accustomed to the change in platelet inventory recovery time, felt the procedures to be "very smooth." There were no donor complaints. No transfusion reactions were reported in recipients of either LRS or control units.

Of the 57 LRS procedures, 5 were unsuccessful: one procedure was terminated after 45 minutes because of donor anxiety, increased pulse and respiratory rate, and pallor. The reaction was interpreted as an anxiety reaction, and there were no medical sequelae. The remaining unsuccessful procedures were terminated because of infiltration at the venipuncture site. The LRS collect concentration monitor flagged 1 (1.9%) in 52 units as potentially containing high numbers of WBCs. This unit contained 4.5 x 10^5 WBCs.

There was no significant difference in processing times for LRS and control procedures or for LRS single- or double-needle procedures (single-needle: 84.9 ± 15.0 min and 80.8 ± 13.6 min, respectively; double-needle: 77.6 ± 14.4 min and 76.9 ± 13.3 min, respectively). There was no significant difference between LRS and control units in final unit volume or platelet yield; nor was there a significant difference between those values in single- or double-needle LRS units (Tables 2 and 3). In contrast, the LRS units had significantly lower WBC counts per unit than the control units (Table 2). A cumulative distribution plot of the residual WBC yield, measured by fluorescence-activated cell-sorting analysis, revealed that data from both the control and LRS units were log-normally distributed and that more than 99 percent of the LRS units contained <1 x 10^6 WBCs, as compared to 50 percent of the control units (Fig. 2). A similar plot showed that 90 percent of the LRS units had a platelet yield >3 x 10^{11} (data not shown). With the flagged unit excluded from the analysis of instrument WBC-reduction capability, the ability of the LRS to achieve a standard cutoff value of <1 x 10^6 WBCs per unit was evaluated by the use of Equation 2. Assuming 99-percent confidence and a 0.5-percent failure rate, the observed mean $\log_{10}$ WBC count (4.51) was less than the calculated maximum acceptable mean $\log_{10}$ WBC value (4.53). Therefore, clinical trial results allowed a 99-percent confidence that 99.5 percent of the components would meet a standard cutoff value of <1 x 10^6 WBCs per unit.

TABLE 2. Summary of Phase II component results

Volume	LRS units	Control units	p values
Number	58	78	
Mean ± SD (mL)	232 ± 19	227 ± 44	0.452
Platelet yield			
Number	58	78	
Mean ± SD (x10^{11})	3.5 ± 0.5	3.5 ± 0.7	0.981
Number with <3 x 10^{11} (%)	6 (10)	4 (5)	
WBCs/unit			
Number	49	44	
Log mean ± log SD	4.5 ± 0.4	5.9 ± 0.7	<0.0001
Median (x10^4)	3.2	81.4	
Range (x10^4)	0.6-45	2.4-4320	

Blood center process evaluation

Process validation. Process validation measurements were performed only by Blood Centers of the Pacific staff members. The first 35 samples from a newly installed LRS instrument were analyzed for platelet yield and residual WBC count by the use of a normal probability plot. WBC data (Fig.

TABLE 3. Results in units collected by LRS single- and double-needle procedures

Volume	Single-needle	Double-needle	p values
Number	14	44	
Mean ± SD (mL)	238 ± 21	230 ± 19	0.231
Platelet yield			
Number	14	44	
Mean ± SD (x10^{11})	3.3 ± 0.8	3.5 ± 0.4	0.127
Number with <3 x 10^{11} (%)	4 (29)	2 (5)	
WBC/unit			
Number	12	37	
Log mean ± log SD	4.4 ± 0.5	4.6 ± 0.4	0.269
Median (x10^4)	1.9	3.5	
Range (x10^4)	0.7-18	0.6-45	

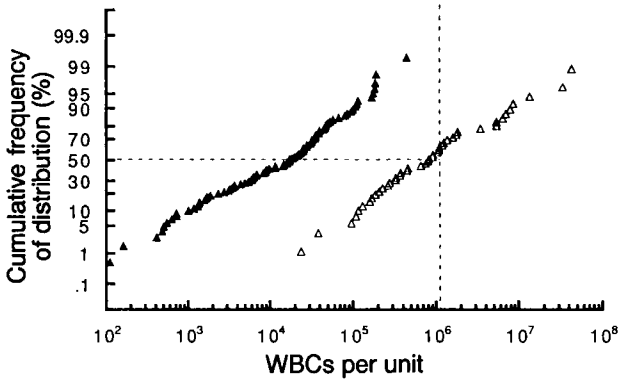

Fig. 2. Phase II clinical trial results: residual WBCs per unit. A log-normal cumulative distribution plot was generated to evaluate the log normality of the distribution of residual WBCs in the LRS and control units, to estimate $\log_{10}$ mean (50th percentiles) and $\log_{10}$ SD (slope of the regression lines), and to demonstrate the percentage of units below the standard cutoff value. Residual WBC values of LRS (n = 49) and control (n = 44) units were log-transformed, assigned a cumulative frequency, and plotted in semi-$\log_{10}$ coordinates. WBC counts were performed by fluorescence-activated cell sorting with a lower limit of detection 0.01 WBCs per µL, allowing linearity analysis to low WBC levels. As the regression lines are linear, the log normality of the distributions is confirmed. All LRS units (▲) contain fewer than 1 x 10^6 WBCs, whereas only 50 percent of control units (Δ) do so.

3A) and platelet yield data (Fig. 3B) were assigned a cumulative frequency, plotted on semi-$\log_{10}$ coordinates, and examined for normality. WBC counts had been obtained with a Nageotte hemocytometer, and, for LRS units, many of the WBC counts were below the detection limit (0.1 WBC/μL). By weighting these truncated data points on the normal probability plot but excluding them from a regression line, the mean and SD could be estimated by a regression algorithm and probit transformation as described by Dumont et al.[14] The mean $\log_{10}$ WBC count and the mean of the platelet yield were 4.0 and 4.03 $\times 10^{11}$, respectively. Finally, the percentage of data points less than the specified limits (standard cutoff value) was inferred directly from the normal probability plot. As shown in Fig. 3A, approximately 99 percent of the units collected with the LRS contained $<1 \times 10^6$ WBCs per unit. As shown in Fig. 3B, 90 percent of the units contained $>3 \times 10^{11}$ platelets per unit.

Ongoing process control

The long-term stability of the apheresis process was assessed through the use of Levey-Jennings control charts as shown in Figs. 4 and 5. Figures 4A and 4B represent long-term stability of WBC counts, and Figs. 5A and 5B represent long-term stability of the platelet yields. Truncated data (substituting 0.1 WBC/μL for a 0.0 Nageotte hemocytometer count) were used to construct control limits for the mean, SD, and WBC counts. Each group was divided into chronological subgroups of four. Although a different subgroup size might have been chosen, four samples represent the currently recommended minimum monthly quality control size, based on the May 29, 1996, Food and Drug Administration memorandum. The mean and SD of each subgroup were calculated. The first graph for each type of analysis (Figs. 4A and 5A) tracks the mean count as a function of subgroup. The second graph for each type of analysis (Figs. 4B and 5B) tracks the SD as a function of the subgroup. By interpreting the charts for nonrandom patterns according to published control chart criteria, one can assess any variations from the natural stability of the process. No shifts in the process were observed. One WBC count was excluded from analysis, as the LRS instrument had already flagged it as an outlier.

Capability assessment and conformance with standards

Capability assessment was performed on 36 consecutive units. Dumont et al.[14] provided a method by which to determine a capability analysis, by using a k_7 factor that corrects for uncertainty in the mean and SD. For WBCs, the specified standard cutoff value was 5×10^6 WBCs per unit. Substituting the $\log_{10}$ standard cutoff value (6.7) and a $\log_{10}$ SD of 0.76 into Equation 2 yields

$$\text{maximum acceptable mean } \log_{10} \text{WBC value} = 6.7 - (0.76 \times k_7).$$

Using a k_7 factor of 3.6 for 90-percent confidence and a 1 percent nonconformance rate, a maximum acceptable mean $\log_{10}$ WBC value of 4.0 was computed. Because the observed mean $\log_{10}$ WBC value was 4.0, we concluded with 90-percent confidence that ≥99 percent of the units will contain $<5 \times 10^6$ WBCs. A similar analysis was performed for platelet yield. We concluded with ≥99.9-percent confidence that ≥75 percent of the platelets collected would exceed the standard of 3×10^{11} platelets per unit.

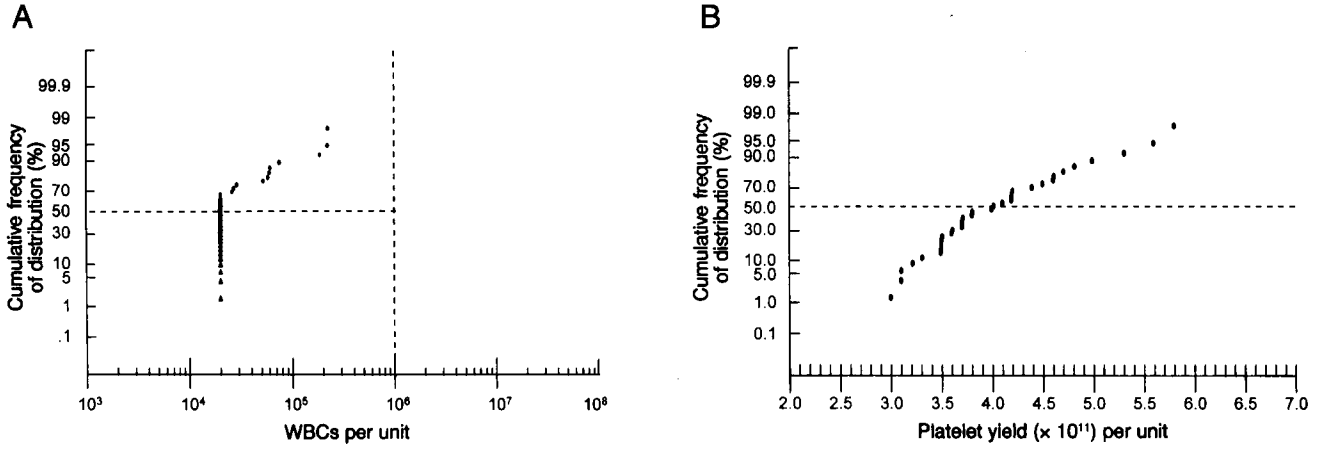

Fig. 3. Local validation results. A) Residual WBC values of LRS units (n = 35) were analyzed with a normal probability plot as described in Fig. 2. However, counts were performed by using a Nageotte hemocytometer with a lower limit of detection of 0.1 WBCs per μL. Therefore, observations reported as "no WBCs seen" (▲) were assigned a cumulative frequency but were excluded from regression analysis. The remaining 12 data points show good linearity, which is indicative of normality. More than 99 percent of the LRS units were found to contain $<1 \times 10^6$ WBCs. B) Platelet yield values of LRS units (n = 35) were assigned a cumulative frequency percentage and analyzed with a normal probability plot. The data are linear, and approximately 90 percent of the LRS units contain $>3 \times 10^{11}$ platelets.

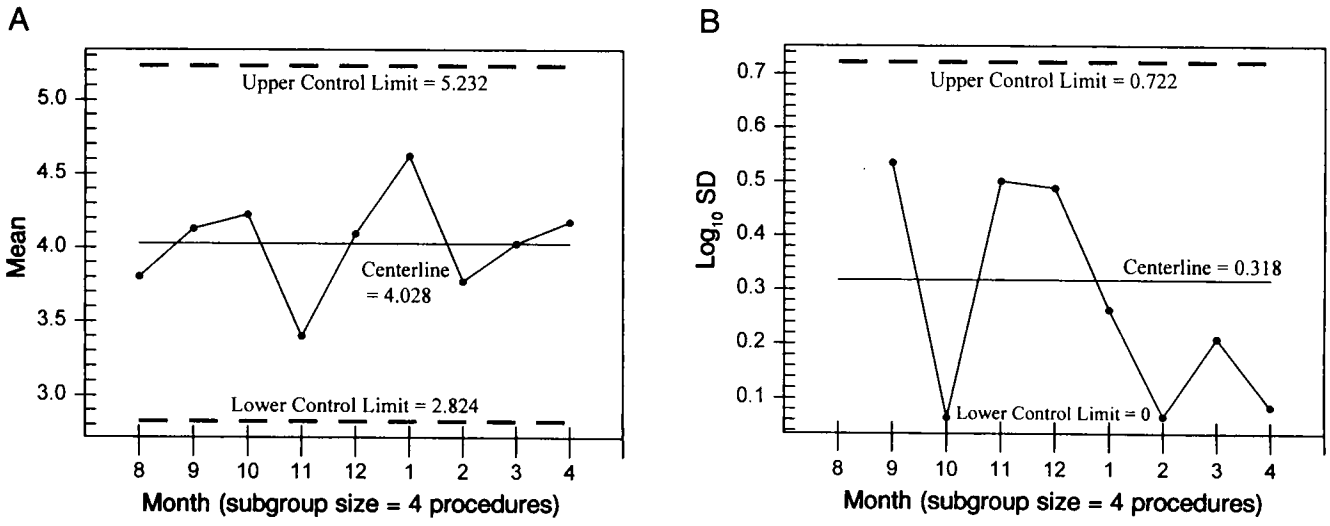

Fig. 4. Ongoing process control: residual WBCs per unit. A) Mean $\log_{10}$ WBC count chart. Long-term stability of the process was monitored by the use of a Levey Jennings control chart. Chronological stability of the mean $\log_{10}$ WBC count is plotted as a function of the corresponding month. The vertical axis represents the mean $\log_{10}$ for a monthly subgroup of four units (only 3 data points were obtained in October). Observations reported as "no WBCs seen" by Nageotte hemocytometer were rounded up to the minimum non-zero WBC count (a $\log_{10}$ transformation approx. equal to 4.4). No nonrandom patterns were observed, which indicates a stable process. B) Log_{10} SD of WBC counts chart. Long-term variability of the process was monitored by the use of a Levey-Jennings control chart. Chronological variability of the $\log_{10}$ SD of the log-transformed WBC count is plotted as a function of the corresponding month. The vertical axis represents the SD of the four log-transformed WBC counts for each month. No nonrandom patterns were observed, which indicates good consistency of the process over time.

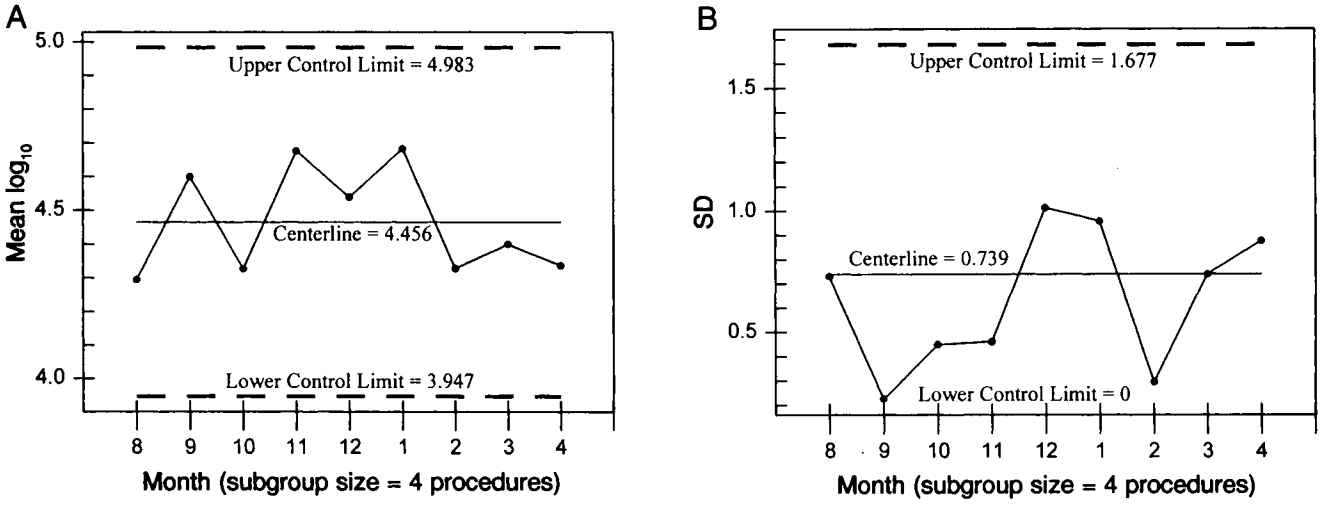

Fig. 5. Ongoing process control: platelet yield per unit. A) Platelet average chart. Long-term stability of platelet yield values was monitored by the use of a Levey Jennings control chart as described in Fig. 4A. No nonrandom patterns were observed, which indicates a stable process. B) Platelet SD chart. Long-term variability of platelet yield values was monitored by the use of a Levey Jennings control chart as described in Fig. 4B. No nonrandom patterns were observed, which indicates little variability in the process over time.

DISCUSSION

These Phase I and II clinical trials have demonstrated that the LRS instrument and software changes improve the performance of the Spectra apheresis system without altering overall safety or patient comfort. Phase I showed that the platelets harvested with the LRS are equivalent in in vitro function and survival to those harvested with the previous version, the Spectra Version 4 instrument. Phase II extended the Phase I results; the LRS instrument produced platelet yields and unit volumes equivalent to those with the Spec-

tra Version 4 during both single-needle and double-needle draws, and it was superior in decreasing residual WBCs per unit. All Phase II LRS units contained $<1 \times 10^6$ WBCs.

A large sample was used in the Phase II studies with the goal of detecting a failure rate of ≤1 percent with ≥95-percent confidence. Capability assessment of the trial data revealed with 99-percent confidence that ≤0.5 percent failure was present: more than 99.5 percent of LRS components would contain $<1 \times 10^6$ WBCs. In fact, the population mean was so much lower than the standard cutoff value that the same degree of confidence and tolerance existed for a cutoff value of 6×10^5 WBCs. The acceptable confidence and tolerance levels differed for the local validation study. Here, the goal was to achieve ≥90-percent confidence of detecting ≤5-percent WBC failure. Therefore, validation was performed on a smaller sample size. Results of the validation showed the blood center to have 90-percent confidence that 99 percent of the units would be WBC reduced and 99.9-percent confidence that 75 percent would exceed platelet yield standards. The process was found to be stable over time.

Quality assurance may be defined as "establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a components meeting its predetermined specifications and quality attributes."[24(17-64)] The methods outlined by Dumont et al.,[14] including process validation, capability assessment, and ongoing process control, make up a practical system for user testing of manufacturer's claims and initiation of ongoing process control. The claim that more than 99 percent of the LRS platelet units will contain $<1 \times 10^6$ WBCs per unit can be tested locally with both a normal probability plot and a capability assessment. If the normal probability plot shows a normally distributed process that meets the manufacturer's claims, the process is validated. Because the true values of a process are never fully known—that is, the mean and SD are estimates with certain degrees of precision—a tolerance factor can be used to adjust for the degree of uncertainty given the sample size and the desired goals. Capability assessment utilizing tolerance factors can be used to estimate the level of conformance with a standard, to update control charts, to check labeling claims, or simply to provide recent and relevant information about the process.

Benefits of the LRS include the assurance of consistent prestorage WBC reduction and ongoing evaluation of quality. The need for component quality assurance is becoming apparent as data accumulate on the less well controlled environment of the bedside in which WBC reduction is sometimes performed: failure of bedside filtration may be as high as 11 percent.[25] Recent data also indicate that there is an increased incidence of alloimmunization in animals transfused with stored cellular components,[26] as well as an increase in cytokine levels that may lead to febrile nonhemolytic transfusion reactions.[6,27,28] Further advantages of fluidized WBC reduction include the absence of platelet contact with an artificial membrane. A recent report suggests that negatively charged filters may cause the activation of bradykinin in platelet concentrates, possibly increasing the risk of anaphylactoid reactions.[29,30]

On the basis of results of the clinical trial, which was followed by successful local process validation and a capability assessment that agreed favorably with the manufacturer's claims, national standards, and user goals over time, the LRS was found to be an effective and efficient system for collecting WBC-reduced apheresis platelets.

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REFERENCES

1. Heddle N. The efficacy of leukodepletion to improve platelet transfusion response: a critical appraisal of clinical studies. Transfus Med Rev 1994;8:15-28.
2. Saarinen UM, Koskimies S, Myllyla G. Systematic use of leukocyte-free blood components to prevent alloimmunization and platelet refractoriness in multitransfused children with cancer. Vox Sang 1993;65:286-92.
3. Bordin JO, Bardossy L, Blajchman MA. Experimental animal model of refractoriness to donor platelets: the effect of plasma removal and the extent of white cell reduction on allogeneic alloimmunization. Transfusion 1993;33:798-801.
4. Leukocyte reduction and ultraviolet B irradiation of platelets to prevent alloimmunization and refractoriness to platelet transfusions.The Trial to Reduce Alloimmunization to Platelets Study Group. N Engl J Med 1997;337:1861-9.
5. Muylle L, Wouters E, Peetermans ME. Febrile reactions to platelet transfusion: the effect of increased interleukin 6 levels in concentrates prepared by the platelet-rich plasma method. Transfusion 1996;36:886-90.
6. Heddle NM, Klama L, Singer J, et al. The role of the plasma from platelet concentrates in transfusion reactions. N Engl J Med 1994;331:625-8.
7. De Witte T, Schattenberg A, Van Dijk BA, et al. Prevention of primary cytomegalovirus infection after allogeneic bone marrow transplantation by using leukocyte-poor random blood components from cytomegalovirus-unscreened blood-bank donors. Transplantation 1990;50:964-8.
8. Bowden R, Slichter S, Sayers M, et al. A comparison of filtered leukocyte-reduced and cytomegalovirus (CMV) seronegative blood components for the prevention of transfusion-associated CMV infection after marrow transplant. Blood 1995;86:3598-603.

9. Busch MP, Lee TH, Heitman J. Allogeneic leukocytes but not therapeutic blood elements induce reactivation and dissemination of latent human immunodeficiency virus type 1 infection: implications for transfusion support of infected patients. Blood 1992;80:2128-35.
10. Shibata R, Siemon C, Rizvi TA, et al. Reactivation of HIV type 1 in chronically infected chimpanzees following xenostimulation with human cells or with pulses of corticosteroid. AIDS Res Hum Retroviruses 1997;13:377-81.
11. Blajchman MA, Bordin JO. The tumor growth-promoting effect of allogeneic blood transfusions. Immunol Invest 1995;24:311-7.
12. Dzik S, Blajchman MA, Blumberg N, et al. Current research on the immunomodulatory effect of allogeneic blood transfusion. Vox Sang 1996;70:187-94.
13. Fournel JJ, Zingsem J, Riggert J, et al. A multicenter evaluation of the routine use of a new white cell-reduction apheresis system for collection of platelets. Transfusion 1997;37:487-92.
14. Dumont LJ, Dzik WH, Rebulla P, Brandwein H. Practical guidelines for process validation and process control of white cell-reduced blood components: report of the Biomedical Excellence for Safer Transfusion (BEST) Working Party of the International Society of Blood Transfusion (ISBT). Transfusion 1996;36:11-20.
15. Dumont LJ, Dumont DF. Enhanced flow cytometric method for counting very low numbers of white cells in platelet components. Cytometry (Communications in Clinical Cytometry) 1996;26:311-6.
16. Kunicki T, Tuccelli M, Becker G, Aster R. A study of variables affecting the quality of platelets stored at "room temperature." Transfusion 1975;15:414-21.
17. Holme S, Vaidja K, Murphy S. Platelet storage at 22 degrees C: effect of type of agitation on morphology, viability, and function in vitro. Blood 1978;52:425-35.
18. Holme S, Holsen H. ADP-induced refractory state of platelets in vitro. I. Methodological studies on aggregation in platelet rich plasma. Scand J Haematol 1975;15:96-103.
19. Fijnheer R, Modderman P, Veldman H, et al. Detection of platelet activation with monoclonal antibodies and flow cytometry. Transfusion 1990;30:20-5.
20. Armitage W. The effects of osmotic stress on human platelets. J Cell Physiol 1985;123:241-8.
21. Feinman R, Detwiler T, Ingerman-Wojenski C. The lumiaggregometer as a research and clinical tool. In: Longnecker GL, ed. The platelets: physiology and pharmacology. Orlando: Academic Press, 1985:429-40.
22. Diem K, Seldrup J. Geigy scientific tables, vol 2. Introduction to statistics, statistical tables, mathematical formulae. Basel: Ciba-Geigy, 1982:52-3.
23. Snyder E, Stack G, Napychank P, Roberts S. Storage of pooled platelet concentrates: in vitro and in vivo analysis. Transfusion 1989;29:390-5.
24. Guideline on general principles of process validation. Center for Drugs and Biologics and Center for Devices and Radiological Health, Division of Manufacturing and Components Quality (HFN-320). Rockville, MD: Food and Drug Administration, 1995:17-61-17-72.
25. Kao KJ, Mickel M, Braine HG, et al. White cell reduction in platelet concentrates and packed red cells by filtration: a multicenter clinical trial. The TRAP Study Group. Transfusion 1995;35:13-9.
26. Blajchman MA, Bardossy L, Carmen RA, et al. An animal model of allogeneic donor platelet refractoriness: the effect of the time of leukodepletion. Blood 1992;79:1371-5.
27. Heddle NM, Klama LN, Griffith L, et al. A prospective study to identify the risk factors associated with acute reactions to platelet and red cell transfusions. Transfusion 1993;33:794-7.
28. Aye MT, Palmer DS, Giulivi A, Hashemi S. Effect of filtration of platelet concentrates on the accumulation of cytokines and platelet release factors during storage. Transfusion 1995;35:117-24.
29. Takahashi T, Abe H, Nakai K, Sekiguchi S. Bradykinin generation during filtration of platelet concentrates with a white cell-reduction filter (letter). Transfusion 1995;35:967.
30. Colman RW, Scott CF, Brandwein H, Whitbread J. More on kininogen measurements in platelet concentrates that are white cell (WBC) reduced with WBC-reduction filters (letter). Transfusion 1996;36:939-40.

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