Plasma Optimization Guide

Improving Plasma Yields from Whole Blood Donations
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*This guide is not intended to be used as a stand alone document to address specific regulatory requirements at the Blood Establishment.*
Plasma Optimization Guide

Overview
Pall Medical supplies whole blood collection and filtration systems that set the standard of care across the globe. Our customers require the highest level of technological competency to support a higher standard of blood supply to the communities they serve. As the needs of our customers continually change, blood centers and hospitals need to make smart decisions to enhance their ability to maximize the value of the blood transfusion products they produce.

The Plasma Optimization Guide is intended to assist Blood Establishments in determining the best processes within their facility to optimize plasma yields while considering the constraints of quality, cost, and time.

The purpose of this guide is to instruct customers on the various elements that affect plasma yields from whole blood donations. The first section of the guide discusses the principals of blood separation and centrifugation. It defines the terminology used in centrifugation, describes the many elements that impact blood separation using centrifugation, and indicates how to establish optimal centrifugation parameters. The next section reviews whole blood collection and processing to reveal how choices made by Blood Establishments within these processes impact plasma yields. The last section concludes with a discussion of the impact of plasma optimization on workflow and discusses the cost benefit analysis of optimizing plasma yields.
Blood Separation and Centrifugation

There is a relationship between the physical properties of blood; liquid viscosity, particle density, particle size, fraction of dissolved solids and the physical principles of centrifugation (gravitational force applied) that impact separation. Guidelines are provided to improve the efficiency of blood component separation using differential centrifugation.

Introduction

There is only so much plasma that can be obtained from a unit of whole blood. Total plasma volume is determined by the volume of blood collected and the donor percent hematocrit (\% Hct). Figure 1 illustrates the theoretical range of plasma that can be recovered from a unit of whole blood.

Figure 1

Theoretical Total Plasma Volume Dependent on Donor % Hct and Collection Volume

Principles of Blood Separation

Blood separation is accomplished by sedimentation and can be defined as the partial separation or concentration of suspended solid particles from a liquid by gravity. The rate of sedimentation is a function of liquid viscosity, particle density, particle size, concentration of the solution (fraction of dissolved solids), and the force of gravity. Sedimentation rates can be calculated for any particulate fluid using Stokes Law of Sedimentation. This equation states that at any given “g-force”, the rate of sedimentation of a particle is directly proportional to its size and density and relative to the density of the suspension fluid. To accelerate sedimentation, the effect of gravity is amplified using “centrifugal force” provided by a centrifuge and can be many thousand times the force of gravity.

Separation of cellular constituents within blood can be achieved by a process known as differential centrifugation. In differential centrifugation, acceleration force is adjusted to sediment certain cellular constituents and leave others in suspension. During the process of differential centrifugation of blood, the sample is separated into two phases: a pellet consisting of cellular sediment and a supernatant that may be either cellular or cell-free.

Stokes Law of Sedimentation

\[ V_g = \frac{d^2 (\rho_p - \rho_1)}{18 \mu x G} \]

where: \( V_g \) = sedimentation velocity, \( d \) = particle diameter, \( \rho_p \) = particle density, \( \rho_1 \) = liquid density,
\( \mu \) = viscosity of liquid
\( G \) = gravitational acceleration
Standardizing Centrifugation Terminology

Centrifugation terminology can be confusing. Abbreviations such as RPM, RCF, TCF and ACE™ are used to describe centrifugation conditions. As the discussion progresses on plasma yield optimization, it is important to clearly understand common centrifugation terminology. Standardization of centrifugation terminology will simplify the approach used later in this document to optimize centrifugation and improve plasma recovery.

Centrifugation terminology is defined below.

- **Revolutions per Minute (RPM)** – is the rotating speed of the rotor arm within the centrifuge during centrifugation.
  
  Example: 5000 RPM. The centrifuge is spinning at 5000 Revolutions per Minute.

- **g – Force or “g”** – is a unit of acceleration equal to the force of gravity.

- **Relative Centrifugal Force (RCF)** – is the force during centrifugation that moves a particulate away from the center of rotation. It is expressed as multiples of the earth’s gravitational field (g). Since RCF includes rpm in the calculation, it changes as the rpm changes during the centrifugation cycle. During acceleration the RCF increases, when the rpm set point is attained, the RCF is constant and during deceleration the RCF decreases. Therefore, the RCF calculated during the centrifugation cycle reflects the force applied at any particular instant in time. The formula to calculate relative centrifugal force in “g” is as follows:

  \[
  RCF (g) = \left[ \frac{rpm}{1000} \right]^2 \times 28.38 \times \text{radius of rotor (inches)}
  \]

  \[
  RCF (g) = (rpm)^2 \times (1.118 \times 10^{-5}) \times \text{radius of rotor (cm)}
  \]

- **Total Centrifugal Force (TCF)** – is a calculation for determining the total applied centrifugal force over the complete centrifugation cycle and has units of g•s. Whereas RCF is the force applied at a given time in the centrifugation cycle, TCF is the Total Force Applied over the complete time and uses RCF (g) x time (s).

  \[
  TCF (g\cdot s) = \text{RCF (g) x time (s)}
  \]

- **Accumulated Centrifugal Effect**¹ (ACE) – is a calculation for determining the total applied centrifugal force over time and uses speed (RPM) and time (s). This calculation can be programmed on most Sorvall™ centrifuges as the “integrator function” or \[ \int \omega^2 dt \].

While centrifugation protocols are frequently defined as RPM and time, this practice can introduce significant variability from one center to another or one centrifuge to another when trying to reproduce the same process. Expressing the force of centrifugation using RPM alone does not take into consideration radius of the rotor or centrifuge load. The variability of centrifugation can be reduced by standardizing the terms of acceleration to specify the acceleration (RCF: g) or total centrifugal force (TCF: g x s) or accumulated centrifugal effect (ACE) that is to be applied to the sample, rather than specifying revolutions per minute and time.

Calculation of RCF is dependent upon the radius of the centrifuge rotor used. The same centrifuge manufacturer with different rotors can produce different acceleration forces (defined as multiples of “g” or the earths’ gravitational force). An example of the difference in RCF for the same RPM settings is shown in Table 1.
Table 1
Relative Centrifugation Force Values for Various Centrifuge Rotors

<table>
<thead>
<tr>
<th>Centrifuge Manufacturer</th>
<th>Beckman</th>
<th>Jouan</th>
<th>Sorvall</th>
<th>Sorvall</th>
<th>Sorvall</th>
<th>Sorvall</th>
<th>Sorvall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotor Model</td>
<td>JS4.2</td>
<td>KR4.2</td>
<td>H6000A</td>
<td>HBB-6</td>
<td>H12000</td>
<td>HLR-6</td>
<td>H4000</td>
</tr>
<tr>
<td>Rotor Radius (cm)</td>
<td>25.4</td>
<td>28.0</td>
<td>26.1</td>
<td>25.5</td>
<td>29.7</td>
<td>25.8</td>
<td>23.1</td>
</tr>
<tr>
<td>RPM</td>
<td>4200</td>
<td>5009</td>
<td>5139</td>
<td>5029</td>
<td>5861</td>
<td>5080</td>
<td>6483</td>
</tr>
<tr>
<td>Relative Centrifugal Force</td>
<td>5522</td>
<td>7826</td>
<td>7284</td>
<td>7127</td>
<td>8307</td>
<td>7200</td>
<td>13022</td>
</tr>
<tr>
<td>Difference in g-force applied</td>
<td>2090</td>
<td>2304</td>
<td>2144</td>
<td>2098</td>
<td>2446</td>
<td>2120</td>
<td>6539</td>
</tr>
</tbody>
</table>

Table 1 shows the impact of using RCF and RPM nomenclature interchangeably. In this example the assumption that centrifugation of blood components at 5000 RPM is equivalent to an RCF of 5000 x g could subject blood components to elevated g-force and therefore compromise the component quality and the efficiency of component separation.

**RPM ≠ RCF**
RPM does not equal RCF and must not be used interchangeably.

Defining the time of centrifugation is also important in standardizing centrifugation protocols. Time may be defined in minutes of acceleration at a given RCF or it may be incorporated in the final calculation of either total centrifugal force or accumulated centrifugal effect. Both TCF and ACE calculations are used to standardize the total effect of centrifugation and use a calculation of either speed and time (ACE) or RCF and time (TCF) to calculate the area under the curve (AUC). An example using area under the curve to calculate TCF for centrifugation is shown in Figure 2.

**Figure 2**
Calculating Total Centrifugal Force Using Area under the Curve

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>Fraction</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-45</td>
<td>1</td>
<td>78</td>
<td>111</td>
<td>152</td>
<td>416</td>
<td>442</td>
<td>462</td>
<td>480</td>
<td>576</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45-90</td>
<td>2</td>
<td>6548</td>
<td>24024</td>
<td>62486</td>
<td>156251</td>
<td>1320000</td>
<td>99606</td>
<td>38270</td>
<td>13104</td>
<td>13968</td>
<td>576</td>
</tr>
<tr>
<td>Fractional AUC (RCF x time)</td>
<td>1.73 x 10^4 g·s</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
As shown in Figure 2, using AUC to calculate TCF is as simple as multiplying the RCF x time (s) for each incremental fraction (depicted in the graph as fractions 1-9) and adding the individual fractions that make up the curve.

A similar method of using time and RPM to calculate area under the curve and associated ACE settings using the integrator function $\int \omega^2 dt$ is well described. The benefits of using the ACE function are to standardize centrifugation by improving run-to-run reproducibility. The setting compensates for differences in acceleration associated with differences in rotor load, voltage fluctuations, loss of instrument calibration, and environmental factors like extreme temperatures.

**General Overview of Centrifugation for Blood Banking**

There are three differential centrifugation processes used to separate whole blood into blood components for transfusion. These are: soft or light spin, hard or heavy spin, and buffy coat spin processing conditions. The soft spin centrifugation profile uses low centrifugal force (slower speed) to allow slower sedimentation of the lighter constituents in whole blood. The soft spin processing condition produces a plasma component that contains a large fraction of the platelets (platelet-rich plasma) and a softly packed red cell component. The buffy coat layer is located at the interface that separates the platelet-rich plasma from the packed red cells, and contains the majority of the white blood cells as well as some of the larger more dense platelets. The soft spin process is typically used to prepare platelets, packed red cells, and plasma from a whole blood component.

Hard spin component processing uses high centrifugal force (faster speed and longer centrifugation time) and produces compacted red cell product and low cellular content, high volume plasma. In this case, the buffy coat layer contains most of the blood leukocytes and platelets. If at this stage the buffy coat layer is separated from the plasma and red cells and is subjected to another round of soft spin centrifugation, a buffy coat platelet product can be produced as is currently performed in Canada and many European countries. In the United States, the AABB recommends centrifugation conditions for the production of blood components as outlined in Table 2.

**Table 2**

<table>
<thead>
<tr>
<th>AABB Recommended Centrifugation Conditions$^3$ for Component Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Component Prepared</strong></td>
</tr>
<tr>
<td>---------------------------------------</td>
</tr>
<tr>
<td>Heavy Spin</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Light Spin</td>
</tr>
</tbody>
</table>
**Optimizing Centrifugation**

The metric for characterizing optimal centrifugation is percent plasma recovery. Converting plasma volume into a percent standardizes plasma yields and reduces variability associated with donor and process differences.

This section will review TCF calculations and demonstrate how different centrifugation protocols may achieve the same TCF with equivalent plasma recovery. Defining TCF for a centrifugation protocol is the key to achieving optimal plasma yields.

**Total Centrifugal Force (TCF)**

Determining centrifuge parameters is often a choice of whether to spin blood at faster speeds (or RCF) for shorter timed intervals or at lower speeds for longer timed intervals. However, to optimize centrifugation it is more important to select the appropriate TCF for your process. Figure 3 illustrates centrifuge profiles comparing centrifugation at 5000 rpm (7284 g) for 7 minutes versus 4150 rpm (5000g) for 10 minutes. When the area under the curve is calculated, the TCF for each condition is the same (approximately 2.5 x 10⁶ g•s). Therefore, centrifugation at a higher g force for shorter time produces the same TCF as spinning at a lower g force for a longer time.

**Figure 3**

*Centrifuge Profile with Equivalent TCF’s*

How does this relate to total plasma recovery? Many believe that spinning at higher RPM is critical for recovering the maximum volume of plasma. Figure 4 and Table 3 represent the same data in a different format and confirm that regardless of whether using higher RPM for shorter spin times (orange curve) or lower RPM for longer spin times (green curve), the percent plasma recovery is dependent on the total centrifugation force or TCF. **Therefore, Total Centrifugation Force is the key to optimizing centrifugation processes.**
Figure 4

*TCF for Differing RPM Settings and % Plasma Recovery*

![Graph showing TCF for different RPM settings and plasma recovery.](image)

Table 3

*Equivalent % Plasma Recovery at Comparable TCF’s*

<table>
<thead>
<tr>
<th>TCF g*s</th>
<th>RPM and Time</th>
<th>% Plasma Yield</th>
<th>SD</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.09 x 10^6</td>
<td>5000 rpm 4 min</td>
<td>85</td>
<td>1.07</td>
<td>3</td>
</tr>
<tr>
<td>1.11 x 10^6</td>
<td>4150 rpm 5 min</td>
<td>85</td>
<td>1.25</td>
<td>3</td>
</tr>
<tr>
<td>1.72 x 10^6</td>
<td>4150 rpm 7 min</td>
<td>91</td>
<td>1.77</td>
<td>6</td>
</tr>
<tr>
<td>2.02 x 10^6</td>
<td>5000 rpm 6 min</td>
<td>91</td>
<td>1.32</td>
<td>6</td>
</tr>
<tr>
<td>2.42 x 10^6</td>
<td>5000 rpm 7 min</td>
<td>93</td>
<td>0.53</td>
<td>6</td>
</tr>
<tr>
<td>2.65 x 10^6</td>
<td>4150 rpm 10 min</td>
<td>92</td>
<td>0.59</td>
<td>6</td>
</tr>
<tr>
<td>3.71 x 10^6</td>
<td>5000 rpm 10 min</td>
<td>94</td>
<td>0.78</td>
<td>6</td>
</tr>
<tr>
<td>4.11 x 10^6</td>
<td>4150 rpm 15 min</td>
<td>93</td>
<td>0.95</td>
<td>3</td>
</tr>
</tbody>
</table>

Understanding TCF can be invaluable when trying to compare the results from more than one spin condition or when trying to obtain the same results at various facilities using different centrifuge settings or centrifuges.
Brake Settings
The impact of deceleration on sedimentation is perceived to be negligible. However brake setting can significantly impact the red cell plasma interface and should be considered when centrifuge optimization is performed.

The data in Figure 5 show that maximum brake settings disrupt the red cell plasma interface at lower TCF but not significantly at higher TCF.

One-way ANOVA shows a statistically significant difference in percent plasma recovery when maximum brake setting is used with a low TCF (4150 rpm for 7 minutes). No statistically significant difference was observed using maximum brake setting and high TCF (4150 rpm for 10 minutes). The significance of brake settings was consistent regardless of percent hematocrit.

High TCF Settings Allow for Faster Braking

Figure 5
Impact of Brake Settings on Plasma Recovery at Different TCF

![Graph showing the impact of brake settings on plasma recovery at different TCFs.](image)
Physical Properties of Blood and the Impact on Centrifugation

The following characterizes the impact of physical properties of blood: percent hematocrit, plasma viscosity, and temperature on plasma yield.

As previously discussed, sedimentation rate is directly related to the force of acceleration and is influenced by density, diameter and size of the cells being separated, viscosity of the fluid, and fraction of dissolved solids.

Effect of Hematocrit

When considering Stokes Law and the elements that impact sedimentation, it is logical that donor differences contribute to the variability in plasma recovery. Factors like the size, density and shape of cells, as well as the density and viscosity of the fluid fraction all impact sedimentation. While donor to donor variation in cell size, shape, and density must be considered in component production processes, these variables are most relevant when manufacturing platelets from whole blood components. Centrifuge conditions for plasma yield optimization are intended to produce a cell-free supernatant and therefore the factors identified above are less significant.

Donor percent hematocrit is relevant to plasma yield optimization in two ways. Donated whole blood with a low percent hematocrit has more plasma available and the red cells are more easily sedimented. The converse is also true. Figure 6 shows a statistically significant (p<0.05) inverse linear correlation (R^2=0.58) between donor percent hematocrit and percent plasma recovery.

Figure 6

Correlation between % Hematocrit and % Plasma Yield

*Units were selected for % Hct and each centrifuge load contained hematocrits ranging from 34 to 43%. TCF applied during centrifugation was 2.5 x 10^6 g*s.
Effect of Temperature

As temperature decreases, plasma viscosity increases and red cell deformability decreases. These two factors reduce sedimentation efficiency. This effect can be minimized by centrifugation at higher TCF as shown in Figure 7. The data was collected under controlled laboratory conditions. Field data shown in Figure 8 support the conclusion that the temperature of blood at the time of centrifugation affects plasma yield. Room temperature (RT) stored whole blood centrifugation produced an average of 5% more plasma than cold stored whole blood.

**Figure 7**
**Impact of Temperature of Whole Blood at Centrifugation**

<table>
<thead>
<tr>
<th>Plasma Recovery (mL)</th>
<th>Temperature of Blood at Time of Centrifugation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT</td>
</tr>
<tr>
<td>TCF=2.65 x 10^6</td>
<td>80</td>
</tr>
<tr>
<td>TCF=1.73 x 10^6</td>
<td>100</td>
</tr>
</tbody>
</table>

4150 rpm for 10 min. 4150 rpm for 7 min.

*No statistically significance between RT plasma yield with high or low TCF. Statistically significant difference in cold conditions (paired t-test: p<0.05).*

**Figure 8**
**Impact of Temperature of Blood at Processing**

<table>
<thead>
<tr>
<th>Plasma Recovery Volume (mL)</th>
<th>Temperature of Blood at Time of Centrifugation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT</td>
</tr>
<tr>
<td>200</td>
<td>150</td>
</tr>
<tr>
<td>250</td>
<td>200</td>
</tr>
<tr>
<td>300</td>
<td>250</td>
</tr>
</tbody>
</table>

30 mL increase in plasma recovery

Room Temperature Processing Yields 5% More Plasma
**Optimal Centrifuge Conditions**

The maximum plasma that is obtainable from a unit of whole blood is between 90 and 95% of the total plasma. This conclusion, demonstrated graphically in Figure 9 is evident by the way the upper portion of the TCF curve becomes flat between 90 and 95% plasma recovery at TCF’s greater than $2.4 \times 10^6$. Similarly, when the optimized TCF between $2.4$ and $2.65 \times 10^6$ g•s is used with different temperature and % hematocrit parameters, the maximum plasma yield is between 90 and 95% as highlighted in tan in Figure 9.

*Units were selected for % Hct and each centrifuge load contained hematocrits ranging from 34 to 43%.*
The maximum plasma yield is consistent with the physical principles of sedimentation where the percentage of dissolved solids can limit the sedimentation rate. Once the red cell pellet is sedimented to the point that 90-95% of the plasma is present in the supernatant layer, additional sedimentation is inhibited by the density of the layer of pelleted red cells. For this reason, the risk of compromising red cell and plasma quality by higher centrifugation forces is greater than the perceived gains in plasma recovery.

**Maximum Plasma Recovery is 90 to 95%**

The optimal TCF minimizes the effect of temperature and percent hematocrit on plasma recovery for a broader range of processing conditions. In Figure 10, the green shaded area of the plasma optimization curve represents the target area for maximizing plasma yield. The yellow shaded area indicates good plasma yield but with greater variability as shown by the large standard deviation. The red area of the curve will produce similar plasma yields as the optimized area; however, there are uncharacterized risks of compromising red cell and plasma quality. Increasing TCF into the red zone will not increase plasma yield above 95%.

**Figure 10**

*Optimized TCF for % Plasma Recovery*

<table>
<thead>
<tr>
<th>Centrifuge Settings</th>
<th>Maximum g</th>
<th>TCF g·s</th>
<th>Percent Plasma Recovery ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>4150 rpm 3 min</td>
<td>5000</td>
<td>5.62 x 10^5</td>
<td>71.0 ± 1.7</td>
</tr>
<tr>
<td>4150 rpm 5 min</td>
<td>5000</td>
<td>1.11 x 10^6</td>
<td>84.6 ± 1.3</td>
</tr>
<tr>
<td>4150 rpm 7 min</td>
<td>5000</td>
<td>1.72 x 10^6</td>
<td>91.2 ± 1.8</td>
</tr>
<tr>
<td>4150 rpm 10 min</td>
<td>5000</td>
<td>2.65 x 10^6</td>
<td>91.9 ± 0.6</td>
</tr>
<tr>
<td>4150 rpm 15 min</td>
<td>5000</td>
<td>4.11 x 10^6</td>
<td>93.3 ± 1.0</td>
</tr>
</tbody>
</table>

In conclusion, the optimal centrifugation TCF for maximum plasma yield is between 2.40-2.65 x 10^6 g·s for the broadest range of processing conditions. This optimized TCF range minimizes the variability of processing associated with % Hct, temperature, and brake settings.

**Optimal TCF for Maximum Plasma Recovery is 2.40-2.65 x 10^6 g·s**
Optimizing Whole Blood Collections

Collection Set Choices – How and Why the Collection Set Chosen Matters
This section describes the reasons why a particular whole blood collection system and volume of whole blood collected can have a direct effect on the amount of plasma recovered during component processing steps.

Impact of CP2D/Nutricel® Additive Solution versus CPDA-1 Collection Systems
Blood containers used for whole blood collections have different configurations using anticoagulant-preservatives and additive solutions. Pall Medical manufactures whole blood collection systems with CPDA-1\textsuperscript{a} anticoagulant-preservative, as well as CP2D\textsuperscript{b} anticoagulant-preservative and Nutricel\textsuperscript{c} additive solution (AS-3).

When using a Pall Collection System with CP2D/AS-3 solutions, a greater volume of plasma is removed from the CP2D red blood cells. After plasma removal, the AS-3 solution is added to the CP2D red blood cell bag, which allows for up to 42 days storage.

CPDA-1 red blood cell products are produced by removing plasma from centrifuged whole blood. The volume of the plasma removed will determine the hematocrit of the CPDA-1 red blood cell unit. To ensure the presence of adequate glucose for red blood cell metabolism for up to 35 days storage, a hematocrit of 80% or lower is required\textsuperscript{v}.

Pall’s CP2D/AS-3 Collection Systems yields approximately 48 mL more plasma for each 500 mL whole blood collection and 7 additional storage days when compared to CPDA-1 Collection Systems. In addition, AS-3 does not contain mannitol and can be used for pediatric transfusions.

If your Blood Establishment decided to convert from CPDA-1 to CP2D/AS-3 Collection Systems, there would be one less bag type at the collection sites, within your IT system, and for inventory management.

By converting from CPDA-1 to CP2D/AS-3 Collection Systems, your Blood Establishment could:

- Gain 48 mL additional plasma from each whole blood collection
- Gain 7 additional storage days for each whole blood collection
- Have one less bag type for collection sites, IT systems, and inventory management

\textsuperscript{a}CPDA-1 = citrate-phosphate-dextrose-adenine solution
\textsuperscript{b}CP2D = citrate-phosphate-dextrose-dextrose solution
\textsuperscript{c}Nutricel = AS-3 solution

Impact of Leukotrap® RC System with RC2D Filter versus Leukotrap WB System
Pall provides a portfolio of products for leukocyte reduction. The Leukotrap RC System with the RC2D Filter provides in-line leukocyte reduction of red blood cells. The Leukotrap WB System provides in-line leukocyte reduction of whole blood. While each system has its benefits, red cell filtration has an advantage over whole blood filtration when it comes to plasma recovery.

When using the Leukotrap WB System, leukocyte reduction is performed on the whole blood \textit{before} centrifugation. As illustrated in Figure 11, whole blood and its associated plasma is held up in the WBF Filter during the leukoreduction step and is not recovered after centrifugation. Thus there is an inherent decrease in the volume of obtainable plasma with whole blood filtration.

With the Leukotrap RC System with the RC2D Filter, leukocyte reduction is performed \textit{after} centrifugation and plasma expression. Therefore all of the recoverable plasma is obtained from the whole blood.
Pall Medical’s Leukotrap RC System with RC2D Filter yields more plasma for each 500 mL whole blood collection as compared to the Leukotrap WB System. As shown in Figure 12, using the Leukotrap RC System instead of a whole blood filtration system your Blood Establishment could increase plasma yields by up to 7%. The Leukotrap RC System with RC2D Filter will allow you to obtain maximum plasma yield from whole blood.

Figure 12
Plasma Recovery - WB versus RBC In-Line System

Increase Plasma Recovery By Using a RBC In-line System
Whole Blood Collection Volume

The volume of the whole blood collection directly impacts the amount of available plasma as described in the Blood Separation and Centrifugation section of this guide. Table 3 summarizes theoretical plasma yields at 38% and 54% hematocrit values. Maximum collection volume settings for the system used will yield more plasma.

Table 3
Collection Scale Settings and Estimated Plasma Recovery

<table>
<thead>
<tr>
<th>Collection Scale Setting (mL) with Leukotrap RC System</th>
<th>Estimated Additional Plasma (mL) with 38% Hct</th>
<th>Estimated Additional Plasma (mL) with 54% Hct</th>
</tr>
</thead>
<tbody>
<tr>
<td>450</td>
<td>279</td>
<td>207</td>
</tr>
<tr>
<td>485</td>
<td>301</td>
<td>223</td>
</tr>
<tr>
<td>500</td>
<td>310</td>
<td>230</td>
</tr>
<tr>
<td>510</td>
<td>316</td>
<td>235</td>
</tr>
<tr>
<td>525</td>
<td>326</td>
<td>242</td>
</tr>
</tbody>
</table>

Automated Blood Scale/Mixer for Accuracy and Standardization

Whole blood units can be simultaneously weighed and agitated during collection by utilizing automated blood collection mixers. These devices are able to standardize collection volumes by ensuring final whole blood units are within 0-2% of the weight or volume setting. By standardizing whole blood collection volumes, maximum settings can be used with less concern for overweight blood collections.

Optimizing Plasma Recovery from Pall Whole Blood Collection Systems

The following describes how to optimize plasma recovery within the individual whole blood collection and component processing steps.

Plasma yield can be optimized by implementing specific centrifuge conditions and by choosing a collection system(s) that will fit into your Blood Establishment’s workflow. There are additional collection and component processing steps that can increase the amount of plasma obtained from each whole blood unit. Using the processing steps recommended below, up to 9.3 mL of additional plasma could be recovered without increasing the collection volume. The steps described in this section are summarized in Table 4.

Table 4
Collection and Component Processing Steps that Yield Additional Plasma

<table>
<thead>
<tr>
<th>Process Steps</th>
<th>Estimated Avg. Plasma Yield (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Empty Donor Line Tubing: without segment numbers</td>
<td>5.2</td>
</tr>
<tr>
<td>2. Empty Donor Line Tubing: with segment numbers</td>
<td>4.8</td>
</tr>
<tr>
<td>3. Express Plasma to Wye versus Collection Bag Port (AS-3 systems only)</td>
<td>2.2</td>
</tr>
<tr>
<td>4. Empty Plasma Bag Tubing</td>
<td>1.9</td>
</tr>
<tr>
<td>(leave one segment)</td>
<td>1.6</td>
</tr>
<tr>
<td>(leave two segments)</td>
<td>0.8</td>
</tr>
<tr>
<td>5. Standardize FFP Volume</td>
<td>75-100</td>
</tr>
</tbody>
</table>
1. Empty Donor Line Tubing when using the following Pall Systems:
   - Leukotrap® RC PL System
   - Leukotrap® RC System with RC2D Filter
   - Leukotrap® WB System

After whole blood collection, staff typically strip the donor line tubing into the collection bag, clamp the tubing close to the collection bag port and mix by inverting the bag. The donor line tubing is refilled with anticoagulated whole blood from the collection bag. Staff will repeat the process and then seal at a designated location on the donor line tubing.

**Recommendation:** Instead of refilling the donor tubing line, leave the tubing empty and seal close to the collection bag port.

**Additional Plasma Yield:** The length of the donor line tubing on Pall Systems is approximately 51.4 inches and contains approximately 8.6 mL of whole blood (6 inches of tubing equals ~1 mL of whole blood). Based on a 40% hematocrit, the Blood Establishment will consistently increase plasma yield by 5.2 mL per whole blood collection by leaving the donor tubing empty.

2. Empty Numbered Tubing when using the following Pall Systems:
   - Non-filter CP2D/AS-3 System
   - Leukotrap® WB System
   - Leukotrap® PL System

When using the Pall systems listed above, routine collection and processing steps leave anticoagulated whole blood in the numbered tubing.

**Recommendation:** Prior to centrifugation, strip the numbered tubing into the primary bag and place a temporary clip close to the port of the primary bag. Coil empty tubing and place in the unit bundle for centrifuge bucket loading. Centrifuge, express the plasma and add the AS-3 solution to the red blood cells as usual. Once the red blood cells and additive solution are mixed thoroughly, remove the temporary clip and fill the numbered tubing with the red blood cell/additive mixture.

**Additional Plasma Yield:** The length of the segmented line tubing on Pall Systems is approximately 48 inches and contains approximately 8 mL of whole blood (6 inches of tubing equals ~1 mL of whole blood). Based on a 40% hematocrit, the Blood Establishment will consistently increase plasma yield by 4.8 mL per whole blood collection when filling the numbered tubing with a red blood cell/additive mixture instead of whole blood.

3. Express RBC/Plasma Interface to Wye versus Primary Bag Port:
   **(AS-3 Systems only)**

During plasma expression, the RBC/plasma interface is typically expressed to the primary bag port.

**Recommendation:** Express the RBC/plasma interface to the wye that connects the plasma bag(s).

**Additional Plasma Yield:** The length of the tubing on Pall Systems between the port of the primary bag and the wye is approximately 13 inches and contains approximately 2.2 mL of plasma (6 inches of tubing equals ~1 mL of plasma). The Blood Establishment will consistently increase plasma yield by 2.2 mL per whole blood collection by expressing to the wye instead of the collection bag port.
4. Empty Plasma Bag Tubing into Plasma Bag:
After plasma has been expressed off from the red blood cells, staff leaves the plasma in the entire tubing length and either seals close to the plasma bag port or creates one or two segments.

**Recommendation:** Strip entire plasma bag tubing line into the plasma bag and seal close to the port of the plasma bag.

**Additional Plasma Yield:** The length of the tubing on Pall Systems between the port of the plasma bag and the wye is approximately 11.5 inches and contains approximately 1.9 mL of plasma (6 inches of tubing equals ~1 mL of plasma). The Blood Establishment will consistently increase plasma yield by 1.9 mL per whole blood collection by stripping the entire tubing line, 1.6 mL per whole blood collection if one segment is left and 0.8 mL per whole blood collection if two segments are left.

5. Standardize Fresh Frozen Plasma (FFP) Volume:
When producing FFP units from a collection system with one plasma bag, staff typically express the plasma into the plasma bag post-centrifugation and freeze as per Standard Operating Procedures to produce a FFP unit.

**Recommendation:** Utilize a collection system with two plasma bags. After the plasma is expressed and separated from the red blood cell component, transfer a standardized volume such as 200mL into the empty plasma bag and continue to process as a FFP unit. The plasma bag with the remaining plasma can be processed as per Standard Operating Procedures for further manufacturing.

**Additional Plasma Yield:** Depending on the hematocrit of the whole blood donor, plasma yields can be up to 75 – 100 mL per whole blood unit collected.
Impact of Plasma Optimization on Work Flow

A number of recommendations have been presented in this guide to improve plasma yield and many of these may impact workflow within Blood Establishment operations. Increasingly Blood Establishments are adopting Lean Six Sigma quality improvement initiatives to streamline their processes as they compete for limited resources with increasing operational costs. The following provides an overview of how to use Lean Six Sigma principles to assess and improve blood center operations for increased plasma recovery.

Selecting a quality improvement initiative with Lean Six Sigma begins with a high level process diagram. An example is a SIPOC diagram that defines Suppliers, Inputs, Process, Output, and Customers as shown in Figure 13. The purpose of the SIPOC diagram is to characterize or define the process and variables that contribute to the final product quality. In this example for blood component production, the Suppliers, Inputs and Process contribute to the quality of the final Output (plasma and LR blood components). The voice of the customer also defines the quality criteria for the final product, such as if the customer is a plasma vendor, additional criteria for quality may be cell-free plasma with a minimum volume of 200 mL.

Figure 13
SIPOC Process Diagram

![SIPOC Diagram](image-url)

There are three areas of focus to improve plasma volume recovery; these include Suppliers, Inputs and Process when using this sample SIPOC.

Supplier choice can impact product quality (Outputs) but for the purpose of this discussion the focus will be on Input and Process variables. Input and Process variables have been previously defined and are summarized in Table 5 and Table 6.
Table 5

**Input Variables That Impact Plasma Yield**

<table>
<thead>
<tr>
<th>Donors</th>
<th>Blood Systems</th>
<th>Equipment</th>
<th>Staff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent hematocrit</td>
<td>One universal collection system to “lean” inventory management, collection</td>
<td>Standardize collection volume using automated blood collection mixers</td>
<td>Empty donor line tubing</td>
</tr>
<tr>
<td>Lower hematocrit improves</td>
<td>and component processing (Leukotrap® RC system with RC2D Filter)</td>
<td>Centrifuge settings: TCF is key! Optimal plasma recovery</td>
<td>Empty numbered tubing</td>
</tr>
<tr>
<td>plasma yield (more female</td>
<td></td>
<td>2.4-2.65 x 10⁶ TCF</td>
<td>Express plasma to wye</td>
</tr>
<tr>
<td>donors)</td>
<td></td>
<td></td>
<td>versus collection bag port</td>
</tr>
<tr>
<td>Higher collection volume</td>
<td>Leukotrap RC System with RC2D Filter provides higher plasma recovery than</td>
<td></td>
<td>Empty plasma bag tubing</td>
</tr>
<tr>
<td></td>
<td>Leukotrap WB System</td>
<td></td>
<td>(leave 2 segments)</td>
</tr>
<tr>
<td>Standardize collection</td>
<td>CP2D over CPDA-1 Collection Systems</td>
<td>Filter vs. non filter during centrifugation has no impact on plasma</td>
<td>Empty plasma bag tubing</td>
</tr>
<tr>
<td>volume</td>
<td></td>
<td>recovery</td>
<td>(leave 1 segment)</td>
</tr>
<tr>
<td>Plasma viscosity</td>
<td></td>
<td>Oval centrifuge buckets improves process with no impact on plasma</td>
<td>Empty plasma bag tubing</td>
</tr>
<tr>
<td>(RT processing improves</td>
<td></td>
<td></td>
<td>leave 2 segments)</td>
</tr>
<tr>
<td>plasma yields)</td>
<td></td>
<td></td>
<td>Standardize FFP volume</td>
</tr>
</tbody>
</table>

Table 6

**Process Step Variables That Impact Plasma Yield**

**Component Production**

1. Optimized TCF
2. Moderate brake setting
3. More RT blood processing (transport)
4. Collect into Leukotrap® RC System vs. Leukotrap WB System
5. Processing Leukotrap RC System vs. Leukotrap WB System
6. AS-3 vs. CPDA-1 System

Once the variables that impact plasma recovery have been identified, assign a metric to benchmark the current process and identify improvement initiatives that will produce the greatest impact on plasma yield with the maximum efficiency. A common metric to define workflow efficiency of blood component processing is time. Table 7 shows process times associated with some recommended component production process steps.
Table 7

<table>
<thead>
<tr>
<th>Process Steps</th>
<th>~ Time (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Optimize TCF</td>
<td>751</td>
</tr>
<tr>
<td>2. Moderate brake setting</td>
<td>Brake 2=386</td>
</tr>
<tr>
<td></td>
<td>Brake 7=195</td>
</tr>
<tr>
<td></td>
<td>Brake 9=149</td>
</tr>
<tr>
<td>3. More RT blood processing (transport)</td>
<td>Variable depending on location and center logistics</td>
</tr>
<tr>
<td>4. Collect into Leukotrap&lt;sup&gt;®&lt;/sup&gt; RC System vs. Leukotrap WB System</td>
<td>0</td>
</tr>
<tr>
<td>5. Processing Leukotrap RC System vs. Leukotrap WB System</td>
<td>150</td>
</tr>
<tr>
<td>6. AS-3 vs. CPDA-1 System</td>
<td>0</td>
</tr>
</tbody>
</table>

When using time as a metric to define workflow efficiency of blood component processing, it is important to consider that component production typically follows a batch mode process and actual time for individual steps may be misleading. Figure 14 provides two different examples of batch mode processing with different timelines due to differences in the batching methods used. In process A, step 1 (blood transport) might be identified as a bottleneck or a rate limiting step since work cannot begin until all the blood is received. In process B where blood transport is continuous and in smaller batches the process workflow follows a more continuous or linear pattern, the overall timeline is shorter as a result of being able to run multiple batch steps earlier and in parallel.

Adding time to the slowest step in a process will have a greater impact on the timeline than adding time to a fast step. In many cases with batch mode processing, additional time can be “absorbed” by the hold time or batch process time for longer steps. There are limitations to using time as a metric for defining efficiency in batch mode processing; however, opportunities exist for adding time with minimal impact on overall timelines if baseline are established and identify bottlenecks and rate limiting steps are identified.

Figure 14

Sample Batch Mode Process Timelines

<table>
<thead>
<tr>
<th>Time Line Process A</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>2 3 4 5 6 7</td>
</tr>
<tr>
<td>2 3 4 5 6 7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time Line Process B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>2 3 4 5 6 7</td>
</tr>
<tr>
<td>2 3 4 5 6 7</td>
</tr>
<tr>
<td>2 3 4 5 6 7</td>
</tr>
</tbody>
</table>

Process Steps
1. Transport units
2. Weigh, Balance, Load Centrifuge
3. Centrifuge
4. Express Plasma
5. Additive Transfer
6. Filter Units
7. Create PRC
Plasma as a Valuable Resource

With the increasing demands for donor resources, a greater emphasis is being placed on maximizing the value of a single whole blood donation. Many blood centers sell recovered plasma for further manufacturing to improve their revenue stream.

Developing a cost benefit analysis may be useful in determining whether all or any of the recommendations for improving plasma recovery are applicable to an individual blood center’s process. When creating a cost benefit analysis, it is appropriate to start with the process improvement steps that provide the highest potential gain with the lowest associated cost. Based on the information discussed thus far, there are four process changes that can result in immediate gains and process efficiency: TCF, room temperature processing, standardized collection set and automated blood collection mixers.

Changes in TCF can provide the greatest gains in plasma recovery with minimal change to process time. Room temperature processing also provides a high gain in plasma recovery (up to 5% additional plasma depending on current TCF) but the logistics and cost of implementing a continuous flow blood transport process will vary from one facility to another. In this instance, a cost benefit analysis may be very helpful in determining whether this process improvement step is cost effective for a particular center.

Standardizing all blood donor collections into a single system can impact the bottom line in a number of ways such as:

- Universal collection system to meet all collection needs
- Simplification of component processing
- Simplification of inventory management
- Streamlining IT

There are also tangible benefits of using automated blood collection mixers. The process flow benefits are:

- Reduction of QNS units
- Reduction of over and underweight units
- Standardization of mixing: fewer clots and gels, less incomplete filtrations.
- Standardization of collection volumes and elimination of the need to balance units prior to centrifugation

In some instances it may be advantageous to create a financial cost model to determine what plasma optimization steps provide the greatest benefit. Additionally, the financial cost model will provide a baseline from which any process improvements can be measured. Factors to consider when making the decision to increase plasma revenue can be broadly categorized as follows:

- Number of whole blood collections per year
- Full Time Equivalent (FTE) wages and benefits
- Technician time (collection and component production)
- Collection and production losses
- Percent of units sold to fractionators
- Estimated plasma volume per unit (sold to fractionators)
- Plasma revenue per liter
Summary

This guide has systematically evaluated the variables that can impact plasma recovery from whole blood collection through component processing and provided recommendations for improving plasma yields. It introduced process workflow and cost benefit analyses to assist the Blood Establishment in characterizing the steps that are expected to maximize plasma gains with minimum effort. Pall Medical Technical Staff is available for assistance or consultation on the elements within this guide. In addition, Pall Medical provides a comprehensive financial cost model for converting to the Leukotrap RC system with RC2D Filter. For further information please contact your Pall Medical Account Manager or visit the Pall Medical web site http://www.pall.com/gotplasma.

1Thermo Application Note: Application Note: AN-LECFBBPROTOCOL-0508.