

Improving Blood Banking Protocols using the Thermo Scientific RC3BP Plus™

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KEY WORDS

- Blood Banking
- Platelet Concentrate Preparation
- Low Speed Centrifuge

Introduction

Blood banks optimize their protocols to yield the highest quality products in the shortest possible time. Optimized protocols may vary from blood bank to blood bank depending on which blood products are most desired. This document is a simple, step-by-step guide to troubleshooting low product yields.

Whole blood collection volumes (405 – 495 mL for 450 mL bags; 450 – 550 mL for 500 mL bags) vary, affecting the volume of plasma, red cells and platelets. Donor-to-donor variability leads to platelet concentrate variations, e.g. low platelet donors give lower yields of platelet concentrate. So how does one start to establish protocols with all these known variables?

Preparation of Platelet Concentrate from Platelet-Rich Plasma

Following the calibration of centrifuges for platelet manufacture in the AABB Technical Manual; the AABB suggests the following centrifuge conditions:

First spin: Separates whole blood unit into platelet rich plasma (PRP), buffy coat (WBC's) and red blood cells (RBCs) by centrifugation at $2000 \times g$ for 3 min.

The first centrifugation step for platelet production is critical. If the first spin is too long and hard, platelets will be lost to the buffy coat and the red blood cells resulting in low platelet yields. Conversely, too short a run does not provide enough time to separate an adequate plasma volume from the whole blood. This results in a reduced volume of plasma products and bloody platelets.

Second spin: The first manufacture of the platelet-rich plasma is followed by the second spin at $5,000 \times g$ for 5 min obtaining the platelet concentrate.

During the second spin, platelets are pelleted from the PRP to yield platelet concentrate and platelet poor plasma. In choosing the length and speed of the second spin, platelets should be pelleted tightly, but not to the point of aggregation. If this happens, the platelet yield will appear low because the cells stick together and the unit can not be used. Also, platelet contaminants in the plasma can decrease the antihemophilic factor (AHF) yield; therefore the plasma should be as cell-free as possible.

Preparation of Platelet Concentrate from Buffy Coat

Platelets may also be prepared from buffy coat, a method used mainly in Europe.

First spin: After centrifugation for 7 min at $5000 \times g$, whole blood is separated into red blood cells (RBCs), buffy coat containing the platelets and platelet-poor plasma (PPP). The buffy coat is separated and further processed to obtain a platelet concentrate.

Either single buffy coat or 4 to 6 pooled (blood group compatible) buffy coats are diluted with plasma or an appropriate nutrient solution.

Second spin: The buffy coat or buffy coat pool is then centrifuged for 3 min at $2000 \times g$ to concentrate the platelets.

Leukocyte-depleted platelets can be prepared by filtration, pre-storage leukocyte depletion is recommended (preferably within 6 hours after recovery). Careful

optimization of the centrifugation conditions allow leukocyte-depleted platelets to be produced by the buffy coat method.

Why Use the Thermo Scientific Sorvall RC3BP Plus

The Thermo Scientific Sorvall RC3BP Plus centrifuge improves productivity for low-speed blood bank applications. Also, the RC3BP Plus centrifuge offers a proven set of swinging-bucket rotors that meet the specific needs of the blood banking market. A summary of centrifuge conditions using Thermo Scientific instruments for the preparation of platelet concentrate from platelet-rich plasma is listed in Table 1.



Thermo Scientific H-6000A swinging-bucket rotor



Thermo Scientific Sorvall RC3BP Plus centrifuge

Rotor	RCF (x g)	Speed (rpm)	Time (min)
<i>1st Spin:</i>			
H-6000A	2,000	2,600	3
HBB-6	2,000	2,650	3
HLR-6	2,000	2,635	3
<i>2nd Spin:</i>			
H-6000A	5,000	4,150	5
HBB-6	5,000	4,200	5
HLR-6	5,000	4,165	5

Table 1. Centrifuge conditions for the first and the second spins for platelets production when using Thermo Scientific Sorvall RC3BP Plus with either H-6000A, HBB-6 or HLR-6 rotors. Processing temperature range: $22 \pm 2^\circ\text{C}$.

Multiple Thermo Scientific Rotors for Blood Banking

The **H-6000A Swinging-Bucket Rotor** offers flexibility for multiple protocols. It accommodates up to 6 single, double, triple or quad blood bags. There is a wide range of adapters for blood collection tubes, conical tubes and bottles.

The **HBB-6 Swinging-Bucket Rotor** can be used to process a wide range of blood bag types. It accommodates up to 6 whole blood bags, in configurations from singles to quintuples. There is a large choice of adapters for up to 12 platelet-rich plasma and buffy-coat bags.

The **HLR-6 Swinging-Bucket Rotor** incorporates a separate filter holder to preserve blood bag integrity. It was optimized for soft-spin production of leuko-reduced packed red cells and platelet concentrates. The HLR-6 accommodates up to 6 triple, quad or quint blood bags with in-line filters.

How to Adjust Your Protocol

Make adjustments in the speed by 200 rpm increments or time by 30 seconds. Adjust the protocol until the desired yield of products is obtained.

- If the platelet yield is low or plasma volume high, decrease the time or lower the speed for the first spin.
- If the plasma yield is low, increase the time or speed for the first spin.

More details are described on the Table 2 “Troubleshooting Guide to Improve Blood Product Yields”.

How to Maximize Blood Product Yields

In slow start/stop mode, you can select from 11 acceleration and deceleration profiles to customize results for maximum yields with minimal re-suspension.

How to Improve Blood Product Reproducibility

As the first centrifugation step for platelet production is critical, to ensure run-to-run reproducibility use the Accumulated Centrifugal

Effect (ACE[™]) function, a standard feature of RC3BP Plus. This function assures you of reproducible blood product yields, regardless of rotor loading. The ACE function calculates the area under the Speed/Time curve as shown in Figure 1. This is the Accumulated Centrifugal Effect experienced during a centrifugation run, by particles in a solution, such as platelets in whole blood.

Using the ACE Mode of Sorvall RC3BP Plus

ACE automatically compensates for variations in acceleration due to full or partial rotor loading by adjusting the centrifuge run time.

An ACE value and speed is set at the start of a run, allowing the centrifuge to control the Accumulated Centrifugal Effect up to deceleration (see Figure 2, 2,800 rpm and an ACE value of 1.60×10^7). As observed in the graph below, times were adjusted to achieve the same overall g-force regardless of the rotor load.

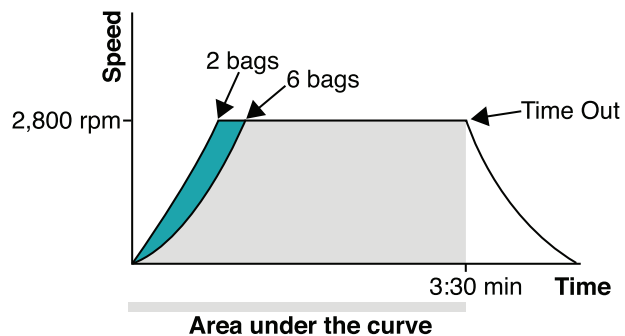


Figure 1. Speed/Time Curve illustrates a typical first centrifugation step (2,800 rpm) with a two-bag rotor load versus six. Notice the two-bag load attains set speed faster than the six-bag load. In a time mode, since both loads will time out at the set time of 3:30 minutes, they experience different gravitational forces (g-force). In order to obtain the same overall g-force for both loads, change the time for 2 bags to 3:00 minutes or use the ACE function.

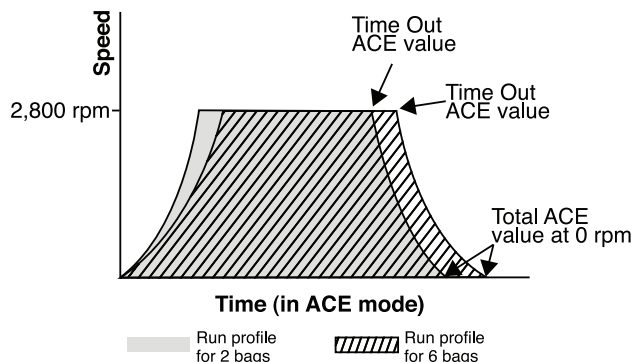


Figure 2. Speed/Time Curve with ACE function

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

Problem/ Observation	1st Spin Finding	1st Spin Action	2nd Spin Finding	2nd Spin Action
Platelet pellet appears firm, well packed	OK	Keep speed and time as is	OK	Keep speed and time as is
Platelet concentrate has aggregates present	OK	Keep speed and time as is	Too hard	Decrease time or speed
Platelet pellet appears soft, loosely packed	OK	Keep speed and time as is	Too soft	Increase time or speed
Plasma and red cell volume acceptable	OK	Keep speed and time as is	OK	Keep speed and time as is
Plasma volume high, red cell volume low	Too hard	Decrease time or speed	OK	Keep speed and time as is
Plasma volume low	Too soft	Increase time or speed	OK	Keep speed and time as is
Platelet yield and plasma volume acceptable	OK	Keep speed and time as is	OK	Keep speed and time as is
Platelet yield is low and pellet appears firm	Too hard	Decrease time or speed	OK	Keep speed and time as is
Platelet yield is low and pellet appears soft	Too hard	Decrease time or speed	Too soft	Increase time or speed
Platelet yield acceptable, plasma volume low	Too soft	Increase time or speed	OK	Keep speed and time as is
No distinct red cell and plasma line. 'Bloody interface'	Too hard	Decrease slow stop rate	OK	Keep slow stop rate same

Table 2. Troubleshooting Guide to Improve Blood Product Yields

Conclusion

The Thermo Scientific Sorvall RC3BP Plus centrifuge provides a complete solution for low speed applications in blood bank laboratories, delivering reliable and reproducible results.

References

1. AABB Technical Manual. 12th Edition 1996.
2. Establishing Blood Banking Protocols on the RC3BP and RC12BP. Application Brief # S00052.
3. Blood Component Manufacturing: In-Process Control Using ACE. Application Brief # S00297.

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