Application Note:
ANCFGLARGVOL-1110

High-Throughput Blood Banking and Bioprocessing Applications Using the New Thermo Scientific Sorvall RC 12BP Plus Centrifuge

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KEY WORDS

- Large Volume Sample
- Blood Banking
- Bioprocessing
- Industrial Laboratory
- Low Speed Centrifuge
- Large Capacity Rotor
- 2 Liter Bio-Bottle

Introduction

Advances in blood banking, industrial biotechnology, bio-production and pharmaceutical laboratories have driven the need to process multiple sample batches. This application brief describes diverse solutions to improve efficiency and reproducibility of high-throughput (large-volume samples) and low-speed applications using the new Thermo Scientific Sorvall RC 12BP Plus low-speed centrifuge, high-capacity H-12000 Bioprocessing swinging bucket rotor (cat. no. 77080) and Nalgene 2 L Bio-Bottle.

The new Sorvall® RC 12BP Plus low-speed centrifuge offers high-quality blood product reproducibility and high throughput to meet demanding blood bank processing requirements. When used with the H-12000 Blood processing swinging bucket rotor (cat. no. 77050), the Sorvall RC 12BP Plus centrifuge enables the ability to run up to 12 blood bags or 12 L of sample in a single run, each with 500 mL (maximum 550 mL) collection volume.

Procedure

Blood Bank Laboratories

PROTOCOL 1: 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[1]:

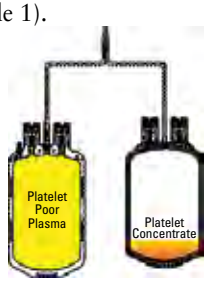
First Spin: Using a soft spin, freshly collected whole blood kept at room temperature is separated to make platelet-rich plasma (PRP), buffy coat (WBCs) and red blood cells (RBCs) by centrifugation at 2000 x g for 3 min at 20 to 22°C.

Typical result after the first hard spin

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.

Note: This separation process must take place within 8 hours of phlebotomy.

Second Spin: To obtain the platelet concentrate, centrifuge the platelet rich plasma using a hard spin also at 20 to 22 °C at 5000 x g for 5 min (see Table 1).



Typical result after the second spin

Thermo Scientific Sorvall RC 12BP Plus Centrifuge

During the second spin, platelets are pelleted from the platelet-rich plasma to yield platelet concentrate and platelet-poor plasma (PPP). 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 cannot 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. Express the platelet poor-plasma on top into the second transfer bag, leaving platelets concentrated at the bottom.

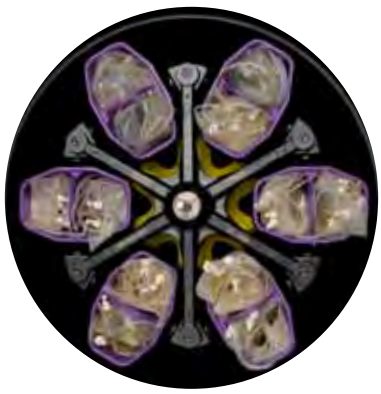
Recommendation: Leave about 50-70 mL of plasma with the platelets concentrate to keep the pH at 6.2 or higher.

	H-12000 Blood Processing Rotor	Recommended RCF (x g)	Recommended Speed (rpm)	Spin Time (min.)	Temperature (°C)
Platelets from	1st spin:	2,000	2,455	3	22
PRP	2nd spin:	5,000	3,880	5	22
Platelet from WBC	1st spin:	5,000	3,880	7	22
(Buffy Coat)	2nd spin:	2,000	2,455	3	22
Cryoprecipitate	1st spin:	4,000	3,471	15	4
from FFP	2nd spin:	5,000	3,880	10	4

Table 1. Centrifuge conditions for blood components production when using a Sorvall RC 12BP Plus centrifuge with a H-12000 Blood processing rotor.
Note: These values are a guideline only. User should test different values to find optimized centrifuge conditions.

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



Thermo Scientific H-12000 Blood Processing Rotor

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 is high, decrease the time or lower the speed of the first spin.
- If the plasma yield is low, increase the time or speed of the first spin. (See Table 2 for a Troubleshooting Guide to Improve Blood Product Yields.[2])

PROTOCOL 2: Preparation of Platelet Concentrate from Buffy Coat (WBC)

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

First Spin: After centrifugation for 7 min at 5000 x g, whole blood is separated into red blood cells, buffy coat containing the platelets, and platelet-poor plasma. The buffy coat is then separated and further processed to obtain a platelet concentrate.

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

Second Spin: To concentrate the platelets, the buffy coat or pooled buffy coats is centrifuged for 3 min at 2000 x g (see Table 1).

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.

Note: For protocols 1 and 2, the platelets should be left stationary to rest for approximately 1 hour at room temperature before being put on a rotator for storage. If not allowed to rest, the platelets may aggregate irreversibly and may not be functional.

Storage of Platelets:

Platelets are stored at room temperature (22 to 24 °C) on a platelet agitator because the gentle agitation will keep the platelets in suspension and prevent clumping. Platelets have a 3-5 days expiration date depending on the collection bag.

Quality Control for Platelets:

- The platelet count must be > 5.5x10 10 platelets per bag in 75% of units tested.
- A minimum of 4 bags must be tested per month.
- The pH of the bag must be 6.2 or greater at the end of the allowable storage period.

PROTOCOL 3: Preparation of Cryoprecipitate

Cryoprecipitate is prepared by thawing fresh frozen plasma (FFP) at temperatures between 1°C to 6°C and then recovering the precipitate.

First spin: preparation of cell-free plasma is prepared from whole blood by hard centrifugation at 4000 x g for 15 min at 4°C. The plasma is frozen at temperatures below -30°C. The frozen plasma is then allowed to thaw at 1°C to 6°C in a water bath or in a refrigerator.

Second Spin: To prepare the cryoprecipitate, the thawed plasma is subsequently centrifuged at 5000 x g

for 10 min at 4°C. The remaining cryoprecipitate should be re-frozen within 1 hour (see Table 1).

Note: Cryoprecipitate contains coagulation Factor VIII:C, Factor XIII, fibrinogen, vWF (Factor VIII:vWF), and fibronectin. It is required for patients with clotting disorders.

AHF	Antihemophilic Factor
PPP	Platelet-Poor Plasma
PRP	Platelet-Rich Plasma
RBC	Red Blood Cells
WBC	Buffy Coat / White Blood Cell

Table 3. Standard abbreviations used in Blood Bank Processing

How To Improve Blood Product

Differences in a full or partial rotor load can cause variations in acceleration times. To ensure quality reproducible separations, choose the Accumulated Centrifugal Effect™ (ACE) function, enter the total Accumulated Centrifugal Effect for the protocol, and the new Sorvall RC 12BP Plus centrifuge will automatically compensate for such variations by adjusting the run time. For additional traceability, our new Thermo Scientific Centri-Log Data Collection Software provides a paperless process tracking and compliance solution.

Nalgene 2 L Bio-Bottle

Industrial Laboratories

To support large batch processing in biotechnology and pharmaceutical environments, the new Sorvall RC 12BP Plus centrifuge, when used with the high-capacity H-12000 Bioprocessing rotor (cat. no. 77080), can spin large volumes with 1 L or 2 L centrifuge bottles. The H-12000 Bioprocessing rotor is capable of processing 6 x 1000 mL or 6 x 2000 mL sample loads in one spin; it can be centrifuged up to 7,340 x g (4700 rpm).

Cell-Culture-Based Vaccine Production

Cell-culture-based technology is robust and reliable and is used in the pharmaceutical industry in vaccine production. Cell culture involves growing Mammalian cells in the laboratory in a nutrient solution. The virus is injected into the cells, and cells and viruses multiply. Then the cells' outer walls are removed and the virus is harvested, purified and inactivated. For cells pelleting, low-speed centrifuge is used at 800 x g for 15 min.

Integrated Solutions for Large-batch Bioprocessing Applications

The new Sorvall RC 12BP Plus low-speed centrifuge and H-12000 Bioprocessing swinging bucket rotor can be combined with the Nalgene® 2 L Bio-Bottle to meet high-volume sample processing requirements. For improved pelleting, the Nalgene 2 L Bio-Bottle is purpose-engineered to fit the unique conical shape of the H-12000 anodized, alumimum swinging bucket rotor. The system greatly increases process efficiency when used with the new Sorvall RC 12BP Plus centrifuge, enabling scientists to process 12 L of sample in a single run.

Nalgene 2 L Bio-Bottles can also be used for gross separation of samples, including bacterial, yeast and tissue isolations.

Sterile, single-use Nalgene 2 L Bio-Bottles are also available.

Conclusion

The new Sorvall RC 12BP Plus centrifuge provides an ideal solution for high-throughput applications in blood banking laboratories, delivering reliable and reproducible results.

When used with the high-capacity H-12000 Bioprocessing rotor and Nalgene 2 L Bio-Bottle, the new Sorvall RC 12BP Plus centrifuge provides scientists in industrial laboratories with an easy-to-use, fully compatible centrifugation solution for accelerated productivity of high-volume sample processing applications.

References

1. AABB Technical Manual. 12th Edition 1996.
2. Schroen, D., Downey, H., and Bailey, J. Establishing Blood Banking Protocols on RC3BP and RC 12BP. Application Brief, S00052. Thermo Fisher Scientific.

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