Blood Banking Applications Using the Thermo Scientific Sorvall BP 8 and 16 and Heraeus Cryofuge 8 and 16 Centrifuges

Romana Hinz, Thermo Fisher Scientific, Osterode am Harz, Germany

Application Note

Key Words
Blood processing, Blood bag systems, Blood bank protocols, Large capacity blood banking centrifuges, HAEMAFlex rotors

Introduction

Blood banks collect, process, store and distribute blood and blood products[1]. After collection, whole blood is separated into its main components: red blood cells (RBCs), platelets and plasma, which are used effectively for patient purpose, while white blood cells are depleted[2]. RBCs transport oxygen to body tissues, platelets help the blood clot, and plasma has specific proteins that allow proper regulation of coagulation and healing[3].

A key instrument in the blood banking workflow is a centrifuge. Centrifuges separate whole blood into RBCs, platelets and plasma. The NEW Thermo Scientific™ Sorvall™ BP 8 and 16 and Heraeus™ Cryofuge™ 8 and 16 blood banking centrifuges provide enhanced capacity of 16 x 500 mL blood bags and user-friendly design, combined with the quick set-up of traceable, reproducible runs for simplified blood processing productivity[4], [5].

This application note introduces the Sorvall BP 8 and 16 and Heraeus Cryofuge 8 and 16 blood banking centrifuges and presents general guidelines to the different protocols for blood component production. This paper gives an overview of possible methods for the preparation of blood components and guidance for the correct use of centrifuge accessories. A troubleshooting guide for the improvement of blood product yields and instruction on how to convert protocols from one rotor to another is also included.

Blood Processing

Blood component preparation is performed to separate blood components from whole blood. RBCs and plasma are produced by a single-step hard spin centrifugation. Platelet concentrates (PC), RBCs and plasma are prepared by a two-step centrifugation. The two main procedures of preparing PCs are either by the platelet-rich plasma (PRP) method or by buffy-coat (BC) method[6].

Platelets from Whole Blood (Buffy-coat (BC) Method)

In European countries, platelets preparation is done by buffy-coat (BC) method[7].

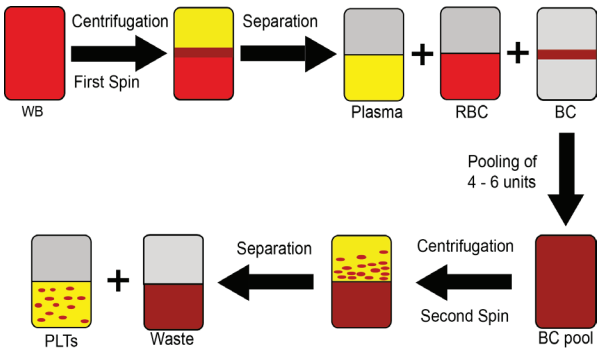

Figure 1: Whole blood processing with the buffy-coat (BC) method.

The first centrifugation step (hard spin) is used initially to separate whole blood into three components: RBCs, plasma and a BC layer. The components are extracted into a so-called, “top and bottom” collection set, in which RBCs and plasma are transferred to storage bags and the BC layer is left in the primary collection bag. This BC layer contains platelets, white blood cells (WBCs), plasma and some RBCs. Components can also be extracted into a top-top blood bag system.

Subsequently, pools of 4-6 ABO-matched BCs are made and either a plasma unit or a platelet additive solution is added.

The second centrifugation step (soft step) is used to produce PCs which are then extracted with or without leukofiltration.

Thermo SCIENTIFIC

Platelets from Whole Blood (Platelet-Rich Plasma (PRP) Method)

Mainly in the United States, platelets are prepared from whole blood by the PRP method[7].

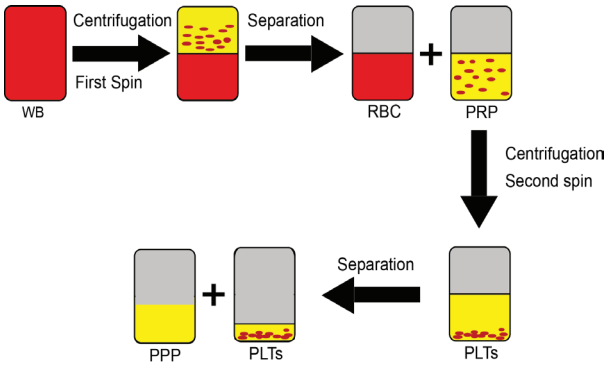

Figure 2: Whole blood processing with the PRP method.

The first centrifugation step (soft spin) results in RBCs and PRP. PRP is extracted with or without leukofiltration into a so-called, "satellite blood bag" and the RBCs are left in the primary bag.

The PRP contains platelets, plasma and WBCs. The secondary hard spin centrifugation produces platelet-poor plasma (PPP) and a platelet pellet. The PPP is extracted into a satellite bag and the platelet pellet is resuspended in plasma.

Red Blood Cells/ Plasma Separation

After a hard spin leukoreduced, whole blood is separated into its two main components: RBCs and plasma[7]. Plasma is extracted into a satellite bag while RBC is left in the primary bag.

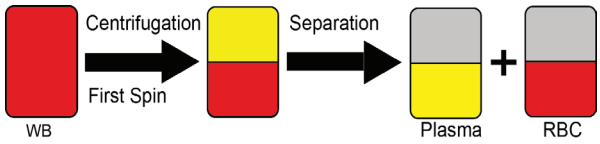

Figure 3: Blood processing with RBC/ plasma separation.

Blood Bank Centrifuge and Accessories

The Sorvall BP 8 and 16 and Heraeus Cryofuge 8 and 16 blood banking centrifuges include innovative features such as energy-saving windshielded rotors with Thermo Scientific™ Eco-spin™ technology, the Thermo Scientific™ Centri-Touch™ user interface and an automatic front-to-back centrifuge door opening and closing with the Thermo Scientific™ Auto-Door™ function and Thermo Scientific™ Auto-Lid™ rotor lid opening. Moreover, not only are 11 acceleration and 12 deceleration profiles provided, but profiles can also be loaded from legacy Thermo Scientific large capacity centrifuges to customize results for maximum yields[4], [5].

When used with the new Thermo Scientific™ HAEMAFlex™ 16 blood processing swinging bucket rotor, the Sorvall BP 16 and Heraeus Cryofuge 16 centrifuges have the ability to run up to 16 blood bag systems in a single run, each with maximum of 500 mL collection volume[8].

Rotor Buckets

Rotor buckets are used to hold blood bag liners. Rotor buckets are also available with the Thermo Scientific™ Dura-Coat™ nickel coating, providing protection against corrosion due to moisture, chemicals or alkaline solutions that otherwise can weaken the structural integrity of a metal rotor bucket.

Blood Bag Liner

The Thermo Scientific blood bag liner enables easy transportation and stabilization of blood bags for processing.

Liner Stand

The Thermo Scientific liner stand simplifies the bucket loading and unloading process, and can help improve the quality of the blood separation.

Balancing Spacers, Weights and Plates

Thermo Scientific spacers are used to compensate low volume blood bags. Thermo Scientific weights and plates are used to counterbalance blood bags with different weight.

Guidelines for Operating Centrifuge Accessories

Blood bags should be packed following the instructions of the blood bag manufacturer [9], [10], [11]. Always observe blood bag manufacturers' specified protocols and recommendations. Before using a new or untested type of blood bag system, it is recommended to perform on-site validation and optimization to predetermine suitability with your particular applications and run parameters.

After packing, blood bags must be placed into a liner. The tubing must be put between the bags with the bag tabs remaining upright to prevent them from becoming tangled around the rotor body during centrifugation.

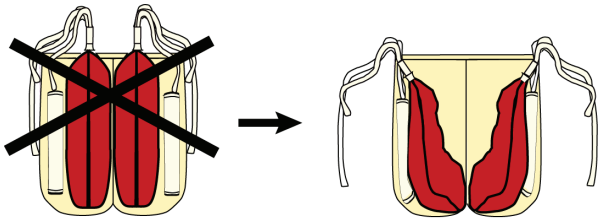

Figure 4: Incorrect loading where tubing was not properly secured.

Blood bags that are not properly loaded can cause rotor failure which could possibly result in leakage or breakage of blood bag systems. Leakage and/or breakage can cause contamination.

Blood bags with a low volume must be compensated by using spacers or balance bags. Otherwise, without compensation, low volume blood bags could result in red cell traps. As balancing bags could easily break after several centrifugation runs, select spacers for use over a longer time period.

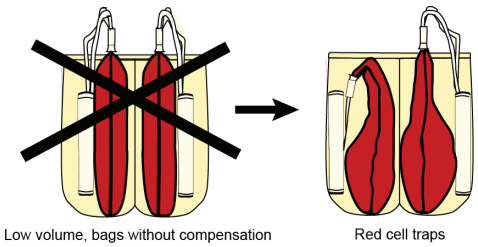

Figure 5: Incorrect loading of bags without compensation.

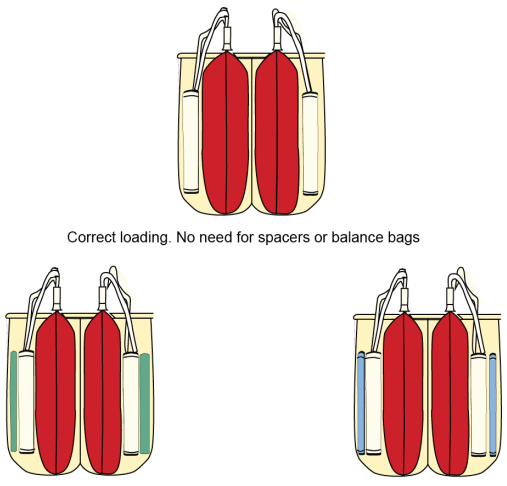

Figure 6: Correct loading.

Optimal Centrifugation Parameters

Blood separation is the partial separation of particles from a liquid by gravity through sedimentation. The rate of sedimentation is a function of liquid viscosity, particle density and particle size, concentration of the solution and the force of gravity. To speed up sedimentation, a centrifuge is used. As a rotor spins in a centrifuge a "centrifugal force" is applied to each particle in the sample; this can be many thousand times the force of gravity *(g)* [12].

The relative centrifugal force (RCF) is the ratio of the centrifugal acceleration at a specified radius and speed to the standard acceleration of gravity. The formula to calculate relative centrifugal force in "g" is as follows:

$$RCF = 1{,}118r\frac{(\mathrm{RPM})^2}{1000^2}$$

where r is the radius in millimeters, and RPM stands for Revolutions per Minute.

At the beginning of the centrifugation process leukocytes and red blood cells (RBCs) sediment more rapidly than platelets. Leukocytes and RBCs have a higher volume (see Table 1). In the middle of the centrifugation process, (depending on centrifugation time and speed) leukocytes and RBCs settle in the lower half of the bag and the upper half contains platelets surrounded by plasma. Then platelets sediment down, due to the centrifugal force, and the leukocytes, situated in the RBCs mass (fluid of higher density), move up.

Table 1: Density and volume of blood components[12].

	Mean Density (g/mL)	Mean Volume (10^{-15} liter)
Plasma	1.026	n/a
Platelets	1.059	9
Monocytes	1.062	470
Lymphocytes	1.070	230
Neutrophils	1.082	450
Red Blood Cells	1.100	87

At the end of the centrifugation process, (depending on centrifugation time and speed) platelet-poor plasma (PPP) is in the upper part of the bag and RBCs at the bottom. Platelets are in the top part of the interface RBCs/ plasma layer, and the majority of leukocytes are in the low part of the interface.

Since there is a relationship between the physical properties of blood and the physical principles of centrifugation that impact separation, the optimal centrifugation for blood component production is achieved by determination of the appropriate centrifuge parameters such as time and speed, and the optimal acceleration and deceleration profile.

Centrifugation conditions for blood component preparation are shown in Tables 2, 3 and 4. These guidelines are based on technical manuals and were validated in the Sorvall BP 8 and 16 and Heraeus Cryofuge 8 and 16 blood banking centrifuges[12], [13], [14].

Table 5 shows a troubleshooting guide to improve blood component production. An adjustment in speed by 200 rpm increments or time by 30 seconds should be done. The protocol must be adjusted until the desired yield of products is obtained.

Table 2: Centrifuge conditions for whole blood processing with the buffy-coat method using the Sorvall BP 8 and 16 and Heraeus Cryofuge 8 and 16 centrifuges.

Method[1]	Thermo Scientific Rotor	Spin	Speed (rpm)	Time[2] (min:sec)	Temperature (°C)	Acceleration Profile	Deceleration Profile
Platelets from WBC (Buffy-coat Method)	HAEMAFlex 6	1st spin:	3744	10:00	22 ± 2	9	4
		2nd spin:	1382	9:30	22 ± 2	3	2
	HAEMAFlex 8	1st spin:	3393	10:00	22 ± 2	9	4
		2nd spin:	1294	9:30	22 ± 2	3	2
	HAEMAFlex 12	1st spin:	3347	10:00	22 ± 2	9	4
		2nd spin:	1282	9:30	22 ± 2	3	2
	HAEMAFlex 16	1st spin:	3201	10:00	22 ± 2	9	4
		2nd spin:	1242	9:30	22 ± 2	3	2

Note: The given values are only a guideline; user should test different values to find optimized centrifuge conditions.
[1] 500 mL blood bag systems.

Table 3: Centrifuge conditions for whole blood processing with the PRP method using the Sorvall BP 8 and 16 and Heraeus Cryofuge 8 and 16 centrifuges.

Method[1]	Thermo Scientific Rotor	Spin	Speed (rpm)	ACE	Temperature (°C)	Acceleration Profile	Deceleration Profile
Platelets from PRP	HAEMAFlex 6	1st spin:	3025	1.70E+07	22 ± 2	9	7
		2nd spin:	3832	5.5 E+07	22 ± 2	9	7
	HAEMAFlex 8	1st spin:	2704	1.70E+07	22 ± 2	9	7
		2nd spin:	3427	5.5 E+07	22 ± 2	9	7
	HAEMAFlex 12	1st spin:	2742	1.70E+07	22 ± 2	9	7
		2nd spin:	3474	5.5 E+07	22 ± 2	9	7
	HAEMAFlex 16	1st spin:	2587	1.70E+07	22 ± 2	9	7
		2nd spin:	3278	5.5 E+07	22 ± 2	9	7

Note: The given values are only a guideline; user should test different values to find optimized centrifuge conditions.
[1] 500 mL blood bag systems.

Table 4: Centrifuge conditions for whole blood processing with the PRP method using the Sorvall BP 8 and 16 and Heraeus Cryofuge 8 and 16 centrifuges.

Method[1]	Thermo Scientific Rotor	Spin	Recommended Speed (rpm)	Time[2] (min:sec)	Temperature (°C)	Acceleration Profile	Deceleration Profile
Red Blood Cell/ Plasma Separation	HAEMAFlex 6	1st spin:	3744	10:00	22 ± 2	9	4
	HAEMAFlex 8	1st spin:	3393	10:00	22 ± 2	9	4
	HAEMAFlex 12	1st spin:	3347	10:00	22 ± 2	9	4
	HAEMAFlex 16	1st spin:	3201	10:00	22 ± 2	9	4

Note: The given values are only a guideline; user should test different values to find optimized centrifuge conditions.
[1] 500 mL blood bag systems.

Table 5: Troubleshooting guide to improve blood product yields.

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 blood cell volume acceptable	OK	Keep speed and time as is	OK	Keep speed and time as is
Plasma volume high, red blood 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 and 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

Conversion to Sorvall BP8/16 and Heraeus Cryofuge 8/16 Centrifuges

As an example, centrifugation conditions of a sample at 3300 rpm in a Thermo Scientific™ Sorvall™ RC 3BP centrifuge with a Thermo Scientific™ HBB-6 swinging bucket rotor with a radius of 258 mm must be adjusted when used in the Sorvall BP 8 (with HAEMAFlex 6 swinging bucket rotor, rotor radius of 255 mm).

Table 6: Maximum rotor radius with the new Thermo Scientific blood banking centrifuges and rotors.

Thermo Scientific Centrifuge and Rotor	Bucket	Rotor Radius Max (mm)
Sorvall BP 8 or Heraeus Cryofuge 8 centrifuge with HAEMAFlex 6 and HAEMAFlex 8 rotor	Single blood bag bucket	255
	Single blood bag bucket with filter pack	258
Sorvall BP 8 or Heraeus Cryofuge 8 centrifuge with HAEMAFlex 8 rotor	Single blood bag bucket	297
	Single blood bag bucket with filter pack	299
Sorvall BP 16 or Heraeus Cryofuge 16 centrifuge with HAEMAFlex 12 rotor	Double blood bag bucket	291
Sorvall BP 16 or Heraeus Cryofuge 16 centrifuge with HAEMAFlex 16 rotor	Double blood bag bucket	316

The conversion is done as follows:
1) Calculate and determine RCF for HBB-6 rotor:

2) Radius in mm

$$\rightarrow RCF = 1{,}118r\frac{(\mathrm{RPM})^2}{1000^2} = 3146$$

So, centrifugation of a sample at 3300 rpm in a Sorvall RC 3BP centrifuge with the HBB-6 swinging bucket rotor will deliver a centrifugal force of 3146 x g.

3) Calculate RPM for HAEMAFlex 6 rotor:

$$\rightarrow RPM = \sqrt{\frac{RCF}{1{,}118r}1000^2} = 3320$$

Centrifugation of the sample in the Sorvall BP 8 with the HAEMAFlex 6 swinging bucket rotor needs to be done at 3320 rpm.

Summary

This application has not only introduced the Thermo Scientific Sorvall BP 8 and 16 and Heraeus Cryofuge 8 and 16 blood banking centrifuges, but also presented different protocols for blood component production using these new centrifuges. It also includes guidance for the correct use of centrifuge accessories, a troubleshooting guide and an instruction of how to convert protocols from one rotor to another.

References

[1] American red cross [online] (2016). http://www.redcross.org/blood.

[2] Higgins, Vl, *Leukocyte-reduced blood components: patient benefits and practical applications*, May; 23 (4); 659 – 67 (1996).

[3] Rodney A. Rhoades, David R. Bell *Medical Physiology, Principles for Clinical Medicine*, 4th edition, (2013).

[4] Thermo Scientific Sorvall BP 8/16 manual, 16.02.2016.

[5] Thermo Scientific Heraeus Cryofuge 8/16 manual, 16.02.2016.

[6] Hans Gulliksson, Platelets from platelet-rich-plasma versus buffy-coat-derived platelets: what is the difference?; *Rev Bras Hematol Hemoter*, 34 (2); 76 – 77 (2012).

[7] Christopher D. Hillyer, *Blood Banking and Transfusion Medicine: Basic Principles & Practice*, 2nd edition, (2007).

[8] Thermo Scientific Sorvall BP 8/16 and Heraeus Cryofuge blood banking centrifuges, brochure, 16.02.2016.

[9] Fresenius. [Online] (21.01.2016). https://www.fresenius.de/689.htm.

[10] Haemonetics. [Online] (21.01.2016). http://wbt.haemonetics.com/en/Products.aspx.

[11] Macopharma. [Online] (21.01.2016). http://www.macopharma.com/category/transfusion/blood-processing/blood-bags/.

[12] Guide to the preparation, use and quality assurance of blood components, European committee (partial agreement) on blood transfusion, 16th Edition (2010).

[13] AABB. [Online] (21.01.2016). https://www.aabb.org/tm/coi/Documents/coi1113.pdf.

[14] Validation was done at Bonfils Blood Center, 717 Yosemite Street, Denver, CO 80230.

thermofisher.com/bloodbankcentrifuges

Australia +61 39757 4300
Austria +43 1 801 40 0
Belgium +32 53 73 42 41
China +800 810 5118 or +400 650 5118
France +33 2 2803 2180
Germany national toll free 0800 1 536 376
Germany international +49 6184 90 6000
India toll free 1800 22 8374
India +91 22 6716 2200
Italy +39 02 95059 552
Japan +81 3 5826 1616
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Nordic/Baltic/CIS countries +358 10 329 2200
Russia +7 812 703 42 15
Spain/Portugal +34 93 223 09 18
Switzerland +41 44 454 12 12
UK/Ireland +44 870 609 9203
USA/Canada +1 866 984 3766
Other Asian countries +852 2885 4613
Countries not listed +49 6184 90 6000

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand

ANCFGBLOODBANK 0216