APPLICATION NOTE

Blood banking applications using the Thermo Scientific Sorvall BP 8 and 16 and Heraeus Cryofuge 8 and 16 centrifuges

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

Key words: *Blood processing, Blood bank protocols, ACE integrator function, Centrifuge loading*

Introduction

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

A key instrument in the blood banking workflow is a centrifuge. Centrifuges separate whole blood into red blood cells, plasma and platelets.

This note presents possible methods for the preparation of blood components and illustrates general guidelines for the different protocols in blood component production. In addition, it provides a troubleshooting guide for the improvement of blood product yields as well as gives guidance on the correct use of centrifuge accessories and explains the Thermo Scientific™ Accumulated Centrifugal Effect (ACE™) integrator function.

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Blood processing

Blood component preparation is performed to separate blood components from whole blood. Red blood cells (RBCs) and plasma are produced by a single-step hard spin centrifugation. Platelet concentrates (PLTs), RBCs and plasma are prepared by a two-step centrifugation. The two main procedures for preparing PLTs are the platelet-rich plasma (PRP) method and the buffy-coat method [4].

Platelets from whole blood (buffy-coat method)

In European countries, platelets preparation is done by the buffy-coat (BC) method [5].

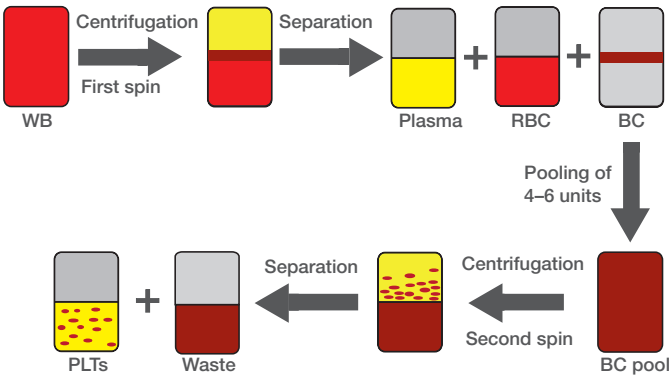

Figure 1. Whole blood processing with the BC method.

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 "top-and-bottom" or a "top-top" blood bag collection set, in which plasma and RBCs are transferred to storage bags and the BC layer is left in the primary collection bag. This BC contains PLTs, white blood cells (WBCs), plasma, and some RBCs.

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 PLTs which are then extracted with or without leukofiltration.

Platelets from platelet-rich plasma (PRP) method

Mainly in the United States, platelets are prepared from whole blood with the PRP method [6].

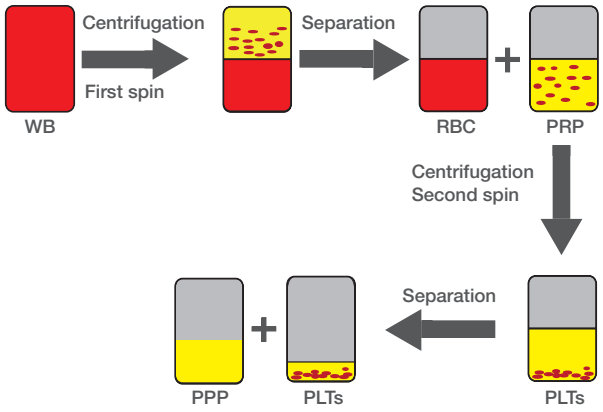

Figure 2. Whole blood processing with the PRP method.

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 re-suspended in plasma.

Red blood cells/plasma separation

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

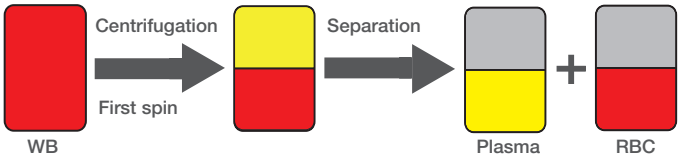

Figure 3. Blood processing with RBC/plasma separation.

Guidelines for blood component production

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.

Since there is a relationship between the physical properties of blood components 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 or ACE with a Thermo Scientific centrifuge, speed and acceleration and deceleration profiles.

Centrifugation conditions for blood component preparation are shown in Tables 1, 2 and 3. These guidelines are based on technical manuals and were validated in the Thermo Scientific™ Sorvall™ BP 8 and 16 and Thermo Scientific™ Heraeus™ Cryofuge™ 8 and 16 blood banking centrifuges [6], [7], [8], [9]. Table 4 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 1. 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 and 500 mL blood bag systems.

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

Note: The given values are only a guideline; user should test different values to find optimized centrifuge conditions.

* At start.

Table 2: Centrifuge conditions for whole blood processing with the PRP method using the Sorvall BP 8 and 16 and Heraeus Cryofuge 8 and 16 centrifuges and 500 mL blood bag systems.

Method	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	9	7
		2nd spin:	3832	5.5 E+07	22	9	7
	HAEMAFlex 8	1st spin:	2742	1.70E+07	22	9	7
		2nd spin:	3474	5.5 E+07	22	9	7
	HAEMAFlex 12	1st spin:	2704	1.70E+07	22	9	7
		2nd spin:	3427	5.5 E+07	22	9	7
	HAEMAFlex 16	1st spin:	2587	1.70E+07	22	9	7
		2nd spin:	3278	5.5 E+07	22	9	7

Note: The given values are only a guideline; user should test different values to find optimized centrifuge conditions.

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 and 500 mL blood bag systems.

Method	Thermo Scientific rotor	Spin	Speed (rpm)	Time* (min:sec)	Temperature (°C)	Acceleration profile	Deceleration profile
Red blood cell/ plasma separation	HAEMAFlex 6	1st spin:	3744	10:00	22	9	4
	HAEMAFlex 8	1st spin:	3393	10:00	22	9	4
	HAEMAFlex 12	1st spin:	3347	10:00	22	9	4
	HAEMAFlex 16	1st spin:	3201	10:00	22	9	4

Note: The given values are only a guideline; user should test different values to find optimized centrifuge conditions.

* At start.

Table 4. 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 cell volume acceptable	OK	Keep speed and time as is	OK	Keep speed and time as is
Plasma volume high and 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 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

ACE integrator function

Obtaining a consistent product requires understanding and controlling process variables. Variations in rotor load, fluctuations in voltage or slight mechanical differences can affect how quickly centrifuges reach set speed. The ACE integrator function calculates the effect of speed in relation to time and adjusts run duration to account for differences in acceleration, thereby improving separation consistency and run reproducibility—run after run, from centrifuge to centrifuge [10].

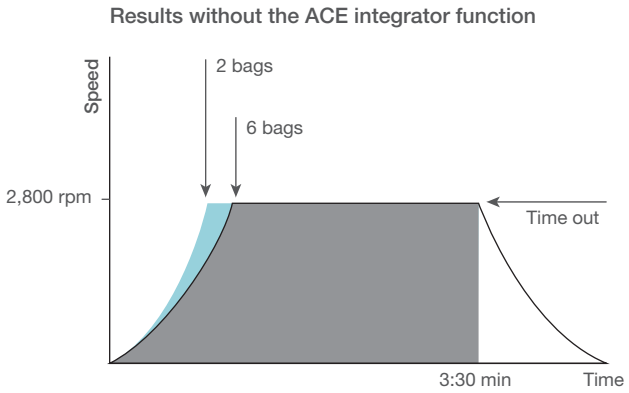

Figure 4. In a typical first centrifugation step, a two-bag rotor load attains set speed faster than a six-bag load. Since both loads will time out at the set time of 3:30 minutes, different total accumulated g-forces are achieved during the run. By using the ACE integrator function, the time for 2 bags would be changed to 3:00 minutes to obtain the same overall accumulated g-force for both loads.

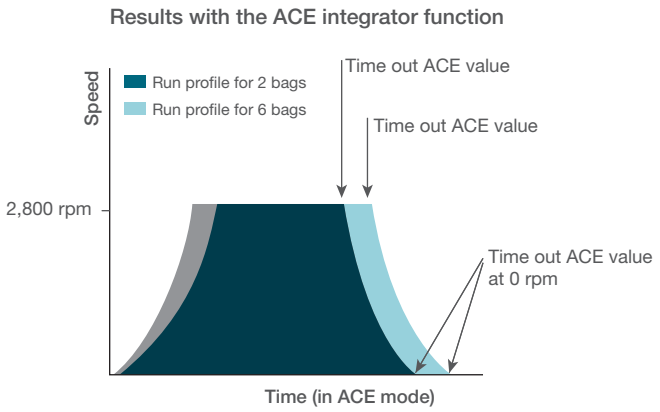

Figure 5. With an ACE value and speed set at the start of a run, times were adjusted to achieve the same overall g-force regardless of the rotor load.

The ACE value is not a calculation as it depends on the acceleration conditions and the deceleration rate. It can be determined by using a stopwatch:

1. Determine the optimal time and speed for you application.
2. Choose a high ACE value.
3. Set your speed at the optimal speed.
4. Set your acceleration/deceleration setting.
5. Start the centrifuge.
6. As the stopwatch reaches the optimal run time, write down the ACE value.

Guidelines for centrifuge loading

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

The following are instructions for properly preparing blood collection systems for centrifugation:

1. Attach all buckets to the rotor and ensure all buckets move freely. All buckets must be in place before run. Choose a centrifugation setting that will achieve the optimal yield for your procedure; See Tables 1–4.
2. When possible, use the ACE integrator function to standardize centrifugation from run to run for better reproducibility and consistency; See section IV.
3. Gently mix the blood bag by inversion.
4. Blood bag systems should be packed following the blood bag manufacturer´s instructions [11], [12], [13].
5. Blood bag systems must be placed into liners. Thermo Scientific liners and liner stands are used for simplifying the liner loading and unloading process. It enables easier transportation and stabilization of blood bags in an effort to improve the quality of the blood separation. Spacers should be used to compensate for low volume blood bags.
6. Counterbalance all liners and use weights as necessary.
7. Place liners into buckets.

8. Make sure all tubing is secured inside the centrifuge bucket. During loading, 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.

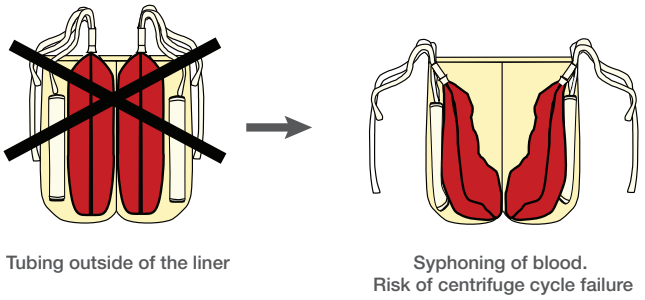

Figure 6. Incorrect loading of tubing not properly secured.

9. Blood bags with a low volume must be compensated by using spacers or balance bags. 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.

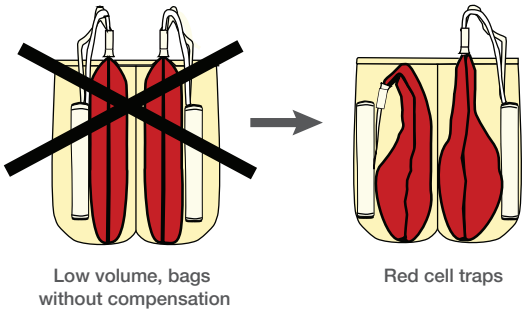

Figure 7. Incorrect loading of bags without compensation.

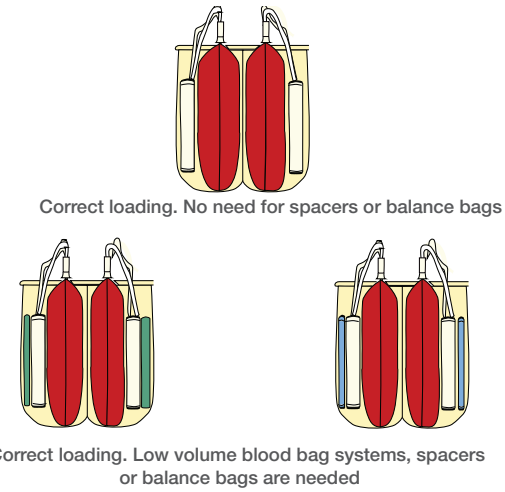

Figure 8. Correct loading.

10. Prior to centrifugation all liners should be loaded with blood bag systems. Never run empty liners. If there is only one blood bag system left, then the empty cavity of the liner should be filled with water filled bags.

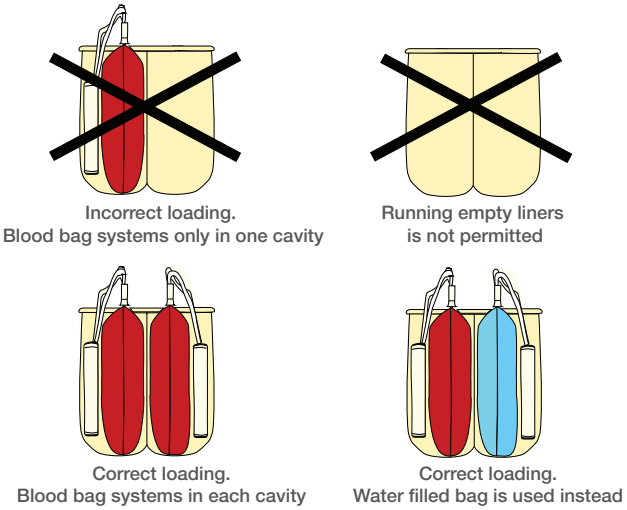

Figure 9. Incorrect loading of only one cavity loaded and correct loading of full liners.

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Summary

This application note presented possible methods for the preparation of blood components and showed general guidelines for different protocols for blood component production. In addition, it provided a troubleshooting guide for the improvement of blood product yields. It also provided guidance on the correct use of centrifuge accessories and explains the ACE integrator function.

References

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