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Faster Isolation of PBMC Using Ficoll-Paque® Plus in the Eppendorf® Centrifuge 5920 R

Nicole Seeligmüller, Eppendorf AG, Hamburg, Germany

Abstract

With its exceptional capacity, high flexibility and speed, the new Centrifuge 5920 R bridges a gap between traditional benchtop centrifuges and standard centrifuges. Its impressive variety of applications is not only reflected by a multitude of applications in the fields of molecular and cell biology, but it provides clear advantages in the areas of clinical diagnostics, for example, during isolation of peripheral blood mononuclear cells (PBMC). This is based on the application of Ficoll-Paque density gradient centrifugation in 15/50 mL conical tubes or blood collection tubes, respectively. Obtaining clean, well-separated PBMCs and therefore a maximum yield of viable cells is essential in keeping sample loss to a minimum. The Multipurpose Centrifuge 5920 R delivered exceptional results for this application even at acceleration/ deceleration rates of 3/3.

Figure 1: Eppendorf Centrifuge 5920 R with Rotor S-4x1000 with high-capacity buckets

Introduction

Human blood consists of equal parts of blood plasma and blood cells. These include erythrocytes (red blood cells), leukocytes (white blood cells) and thrombocytes (platelets). Leukocytes are further subdivided into different cell types. These include, for example, lymphocytes and monocytes, which (in co-operation with other cells) form the basis of the innate immune system and which, owing to their simple nucleus, are referred to as peripheral blood mononuclear cells (PBMC). The term lymphocyte encompasses two major classes, B-lymphocytes and T-lymphocytes. B-lymphocytes are responsible for antibody production, whereas T-lymphocytes produce signal molecules which will lead finally to the removal of diseased or foreign cells. [1]

Lymphocytes are isolated from "buffy coats" (whole blood concentrates without serum). PBMC can be separated from other components of the blood, e.g. erythrocytes and granulocytes, using density gradient centrifugation with Ficoll-Paque PLUS. Ficoll has a density of 1,007 g/mL. Due to their higher density, erythrocytes, granulocytes and dead cells will pass through the Ficoll layer, whereas lymphocytes and monocytes, based on their lower density, will accumulate in the plasma-gradient-phase (figure 2). This approach is concordant with the method for isolation of PBMC, developed by Bøyum in 1968. [2] [3]

Today, countless applications in biomedical research and routine diagnostics rely on highly viable and functionally intact cell populations. Due to uncomplicated and robust feasibility, density gradient centrifugation is ubiquitously applied worldwide.

One prerequisite for a clean PBMC isolation with a maximum yield of living cells is the formation of a clear interphase. For this reason, the procedure must be carried out with the least vibration possible. Usually, a mixing of the phases can only be avoided by centrifugation with the rotor brake deactivated [4], which, constitutes an extremely time-consuming step within this application.

This Application Note will show how the new Eppendorf Centrifuge 5920 R*, in combination with a variety of swing-out rotors, is capable of meeting such high demands. At the same time, the user will enjoy considerable time savings through individual selection of acceleration and deceleration ramps.

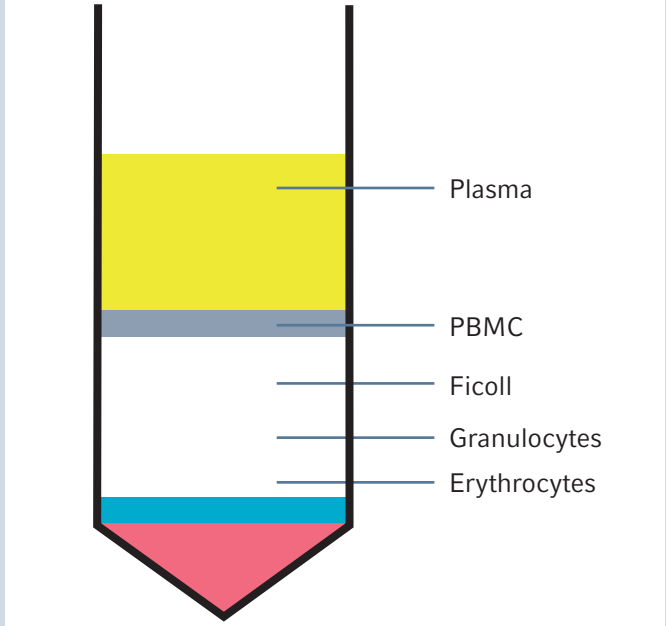

Figure 2: Density gradient of a buffy coat with Ficoll-Paque PLUS (schematic)

Materials and Methods

Materials

Eppendorf Centrifuge 5920 R with the following swing-out rotors:

1. Rotor S-4xUniversal-Large plus adapter 50 mL Conical Tubes
2. Rotor S-4x1000 with high capacity buckets plus adapter 50 mL Conical Tubes
3. Rotor S-4x750 plus adapter 50 mL Conical Tubes (results not shown)

> Eppendorf Conical Tubes 50 mL, Eppendorf
> Eppendorf Research® plus Pipette 1000 µL, Eppendorf
> Eppendorf Easypet® 3, Eppendorf
> Ficoll-Paque PLUS, GE Healthcare® Bio-Sciences AB
> Dulbecco's Phosphate Buffered Saline 1x (DPBS), Gibco® by Thermo Fisher Scientific®
> Buffy Coats, human, University Hospital Eppendorf, Institute for Transfusion Medicine, negative tested for infectious diseases and Herpes viruses (from previous day).
> Serological pipettes 10 mL, Eppendorf
> Wide mouth flask 400 mL, Eppendorf
> Surface disinfectant Bacillol® plus, Bode Chemie®
> Trypan blue 0.4%, SIGMA-ALDRICH®
> Microscope Axio Observer.A1, Zeiss®

*This centrifuge is an in vitro diagnostic accessory and therefore itself is an in vitro diagnostic device according to Directive 98/79/EC of the European Parliament and the council, dated october 27, 1998.

Methods

Ficoll-Paque PLUS-density gradient centrifugation

1. Bring Ficoll-Paque PLUS and PBS to room temperature.

2. Invert Ficoll-Paque PLUS several times.

3. Wipe the blood bag with 70 % ethanol, cut the lower tube and transfer the blood to a wide-mouth flask in a sterile manner.

4. Dilute the blood 1:1 in PBS, close the bottle and mix by careful inversion.

5. Place 15 ml of Ficoll into each of the 50 mL Eppendorf Conical Tubes.

6. Overlay the Ficoll with the blood/PBS mixture using a serological pipette at lowest speed.

TIP: In order to avoid compromising the subsequent purity of the PBMC, mixing of Ficoll and blood must be avoided at all costs. For this reason, the tube should be held at an angle and the blood mixture should be released slowly from the pipette, touching the tube wall, in order to achieve a good overlay.

7. Centrifuge the samples in the desired swing-out rotor for 30 minutes at 400 x *g* and 20 °C, selecting acceleration/deceleration rates of 0/0 or of 3/3**, respectively, with setting "at set rpm".

TIP: In order to effectively prevent mixing of the phases, normally brakes must be inactivated completely. Exact adherence to temperature specifications is also very important as temperature differences will change the density ratios of the liquids and may therefore impact the result of the separation negatively.

8. After completing the centrifugation run, carefully remove the sample tubes to avoid mixing of the phases.

**Possible ramp settings Centrifuge 5920 R: deactivated acceleration or deceleration respectively (0/0) up to fastest acceleration/brake (9/9).

Purification of lymphocytes

1. Carefully aspirate 2/3 of the top layer (containing plasma and platelets) using a sterile serological pipette until the interphase (containing the mononuclear cells) is within reach.

2. Using an Eppendorf pipette, aspirate the entire lymphocyte layer while keeping the volume to a minimum and transfer into a fresh tube.

TIP: During this step, as little Ficoll-Paque PLUS, or supernatant, respectively, should be transferred.

3. Add at least 3 volumes of PBS to the lymphocyte layer and carefully mix by pipetting up and down.

4. Centrifuge for 10 min at 100 x *g* and 20 °C and discard the supernatant.

5. Repeat steps 3 and 4.

6. Resuspend the cell pellet in a medium suitable for downstream applications.

Viability test and determination of yield

1. Dilute cells 1:1 in trypan blue and count.

2. Determine viability and yield.

Results and Discussion

In order to evaluate the quality of the separation, it was determined whether a defined interphase with clearly delineated phase transitions was visible. Turbidity of the liquid may indicate suboptimal separation of the PMBCs. Figure 3 (a-d) shows the results obtained from the density gradient centrifugations carried out in the Centrifuge 5920 R using different swing out rotors. For each rotor, acceleration and deceleration rates of 0/0 and 3/3 were tested. It is evident that optimal separation of blood components was achieved in all cases, and that vortex effects during deceleration of the rotor could be completely avoided. This result is especially striking when compared to other centrifuges which yield less impressive results due to rotor vibration during the run (figure 3e).

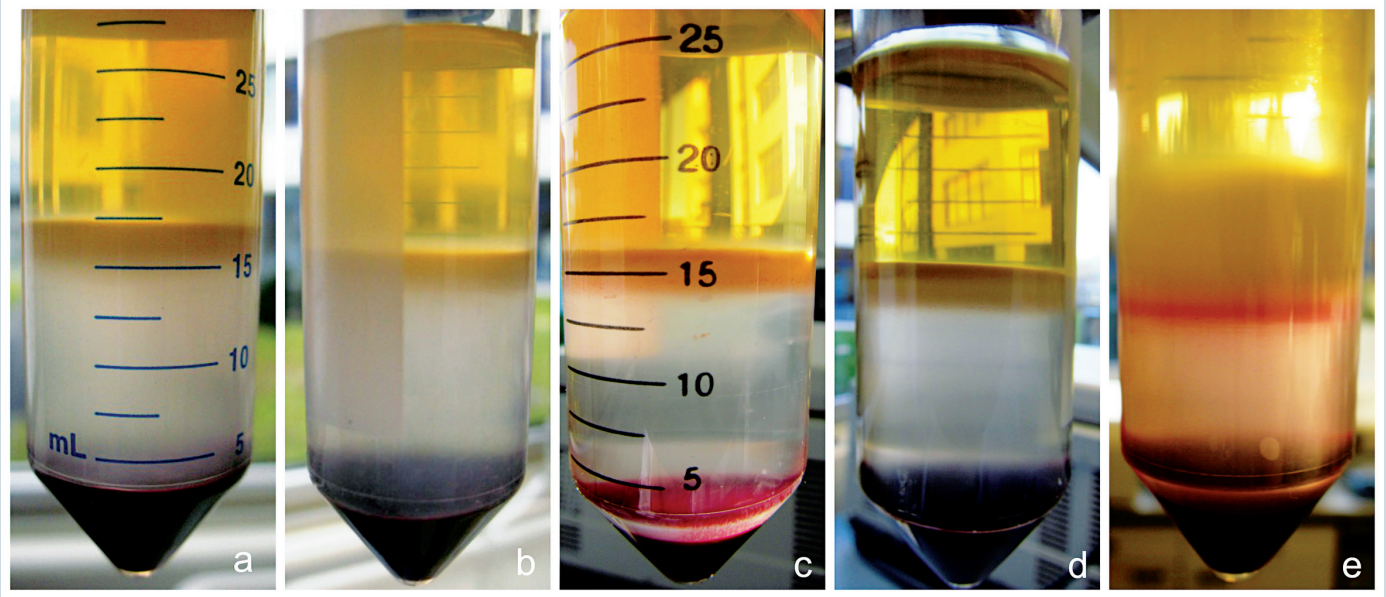

Figure 3: Results obtained after density gradient centrifugation in the Centrifuge 5920 R. A) Rotor S-4x1000 with high capacity buckets (ramp 0/0) B) Rotor S-4x1000 with high capacity buckets (ramp 3/3) C) Rotor S-4xUniversal-Large (ramp 0/0) D) Rotor S-4xUniversal-Large (ramp 3/3) E) Negative example: cloudy interphase, turbid plasma-/Ficoll phase.

In order to confirm the quality of the PBMC isolation using Ficoll-Paque PLUS, in addition to visual inspection of the centrifuged samples, yield and viability of the PBMCs obtained from the rotor S-4xUniversal-Large were analyzed exemplarily. Data provided by GE Healthcare, which routinely achieved a viability of 95 % (+/-5 %) during internal testing, served as a reference [5]. The studies performed at Eppendorf showed an average viability of 94 %, which is in line with expected values.
According to the literature, the expected yield of mononuclear cells falls between 0.8 and 3.2 x 10^6 cells/mL of blood [6].
A result of 3.0 x 10^6 cells/mL (ramp 0/0) or 2.16 x 10^6 cells/mL of buffy coat (ramp 3/3), respectively, places the total yield of viable cells within a very good range.
These results demonstrate that the Centrifuge 5920 R fully meets the demands of low vibration performance, independent of the swing out rotor used, and independent of whether an acceleration/deceleration rate of 0/0 or 3/3 was selected. Furthermore, a ramp of 3/3 allows considerable time savings of up to 19.7 min (36 %) compared to the centrifugation parameters recommended in literature (deactivated brake) (figure 4). [7]

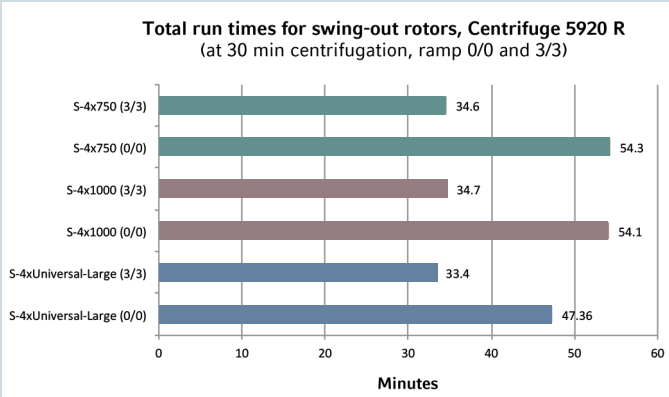

Figure 4: Total centrifugation times for density gradient centrifugation in the new Eppendorf Centrifuge 5920 R using different swing-out rotors and ramp settings 0/0 or 3/3.

Conclusion

In order to obtain a high yield of viable PBMCs, Eppendorf recommends the new Centrifuge 5920 R for density gradient centrifugation using Ficoll-Paque PLUS. According to available test results, consistent high quality of yield can be expected with all available swing-out rotors for 50 mL conical tubes. Its large capacity and the option of significantly reducing the time required for rotor deceleration, Centrifuge 5920 R is also ideally suited for laboratories processing high sample volumes.

Literature

[1] Theml H, Diem H, Haferlach H. Taschenatlas der Hämatologie: Morphologische und klinische Diagnostik für die Praxis. 5th edition, Stuttgart: Georg Thieme Publishers; 2002
[2] Bøyum A. Isolation of mononuclear cells and granulocytes from human blood. Scan. J. Clin. Lab. Invest. 1968, 21 (Suppl. 97): 77-89
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[4] Heine H, Uschkureit T. Software-controlled acceleration and deceleration rates for optimal isolation of mononuclear cells. Eppendorf Application Note 74; www.eppendorf.com
[5] Ficoll-Paque™ PLUS Instructions. www.gelifesciences.com
[6] HIV/AIDS Network Coordination: Cross-Network PBMC Processing Standard Operating Procedure. www.hanc.info
[7] Luttman W, Bratke K, Küpper M, Myrtek D. Der Experimentator: Immunologie, 4th edition, Heidelberg: Springer Publishers; 2014

Ordering information

Description	Order no. international	Order no. North America
Centrifuge 5920 R, refrigerated, 230 V/50 – 60 Hz	5948 000.018	
Centrifuge 5920 R, refrigerated, 120 V/50 – 60 Hz		5948000131
Rotor S-4xUniversal-Large, incl. universal bucket	5895 190.006	5895190006
Adapter 50 mL Tubes	5920 735.004	5920735004
Rotor S-4x1000, incl. high-capacity bucket	5895 118.003	5895118003
Adapter 50 mL Tubes	5920 715.003	5920715003
Rotor S-4x750, incl. 4x750 mL buckets	5895 120.008	5895120008
Adapter 50 mL Tubes	5825 733.002	5825733002
Eppendorf Research® plus, single channel, variable, 100 - 1000 µL, blue	3120 000.062	3120000062
Eppendorf Conical Tubes 50 mL, 50 mL, sterile, Pyrogen-free, DNase-, RNase- and DNA-free	0030 122.178	0030122178
Eppendorf Easypet® 3, inclusive power supply, wall adapter, resting stand, 2 membrane-filters 0.45 µm, 230 V/50 Hz (EU)	4430 000.018	4430000018

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