Software controlled acceleration and braking ramp for optimized isolation of mononuclear cells

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Introduction

Many applications in clinical and cytological laboratories require rapid processing of the samples under examination. The centrifuges required for such applications should therefore be able to accelerate as fast as possible and to brake at a correspondingly fast rate so that temperature-sensitive samples such as culture cells can be used for further steps of the relevant application without remaining in the centrifuge for a long time. With the development of the Centrifuge models 5702, 5702 R and 5702 RH, Eppendorf offers modern laboratory centrifuges for such applications that have extremely short acceleration and braking times (< 25 sec).
Besides speed, another decisive criterion for the quality of a centrifuge in the routine lab is a feature called the re-mixing rate. Therefore, special attention has been paid to the development of a second acceleration and braking ramp that is gentle and yet fast. This function is called a Soft function and enables the user to perform sensitive applications such as density gradient centrifugations to isolate blood cells quickly and reproducibly.
The isolation of human mononuclear cells (MNC) from whole blood is one of these standard methods used in cytological laboratories. This cell population, which contains T- and B-lymphocytes and monocytes, can then be used directly for experiments or serves as a starting population for further isolation of single types of cells.

To isolate MNC by density gradient centrifugation, the main method used is a method based on the density properties of Ficoll (1). Due to their high density, red blood cells (erythrocytes) pass the Ficoll phase (density: 1.077 g/ml) and form a sediment. A high-polymer sugar, Ficoll also causes agglutination (clumping) of the erythrocytes, thus accelerating sedimentation. Lympohocytes, thrombocytes and monocytes collect at the plasma gradient phase (specific weight less than 1.077 g/ml) and can thus be enriched.
The results of the software optimization for the development of an optimum Soft function are summarized below.

Material and methods

1. 10 ml Biocoll separating solution (contains Ficoll®400, Biochrom AG) at room temperature is pipetted into a conical 50 ml tube (Falcon® tubes, BD Biosciences).
2. Human blood is diluted 1:2 with HBSS (Hanks balanced salt solution, Invitrogen).
3. The Ficoll phase is covered with a layer of 35 ml diluted blood. It is essential that blood and Biocoll are not mixed!
4. Centrifugation is performed for 30 minutes at 1600 rpm (440 x g) and with the Soft function switched on and off (Centrifuge 5702, rotor A-4-38, adapter 5702 734.004 for conical centrifuge tubes).
5. Optical analysis of the resulting gradients using a digital camera.
6. Then the HBSS/thrombocyte/blood plasma supernatant is carefully pipetted off to approx. 1 cm above the MNC ring.
7. The MNC is carefully transferred to a fresh 50 ml vessel using a 10 ml pipette. All the steps are performed on ice and no more than 20 ml per vessel is processed.
8. HBSS is used to top up the level to 50 ml. The mixture is shaken to mix it thoroughly and centrifuged with the brake switched on (10 min, 1600 rpm, 440 x g).
9. When the supernatant has been removed by pipette, the pellet is resuspended in 1 ml HBSS and step 7 (centrifugation) is repeated.
10. Finally, the supernatant is removed by pipette and the cells are absorbed by 5 ml culture medium. The number of the cells is counted (Neubauer counting chamber) and their size is determined. For this purpose, 50 µl of the cell suspension is mixed with 10 ml counting fluid (Isoton II, Beckmann Coulter, Krefeld) and analyzed in a Coulter Channelyzer 256 (Beckmann Coulter, Krefeld).

Results and discussion

The results contained in this experiment were initially documented with regard to quality by the visual assessment of the gradients (see step 4, material and methods). At the end of the experiment they were verified with regard to quantity by counting the number of cells. Care was taken that the blood taken from one single donor could be used to form at least four gradients: two in the centrifuge model 5702 being investigated and two in a comparison centrifuge. For this study the comparison model was a Centrifuge 5810 with a swing-bucket rotor A-4-44 and the corresponding adapters for conical 50 ml tubes (5804 758.005). The Centrifuge 5810 has 9 braking ramps and 9 acceleration ramps and the ramp 0 (lowest acceleration and brake) selected in this case is known to produce very good results for this application.
First, the centrifuge 5702 was operated with the brake switched on for all the necessary centrifugation steps. Fig. 1a shows clearly that, besides careful layering of the whole blood and medium mixture on to the Ficoll (see material and methods), the brake setting used in the centrifuge is of great significance for this application. When the brake is switched on, isolation of the mononuclear cells is not possible as no MNC ring can be detected.
In the next step a Soft function was implemented that makes possible slightly prolonged acceleration and unbraked stopping of the rotor (Fig. 1b). Comparison with centrifuge 5810 (Fig. 1c) proves that a detectable gradient was formed in both experiments but that the one formed in the comparison centrifuge was significantly more distinct. This optical impression is confirmed by the cell yield summarized in Table 1.

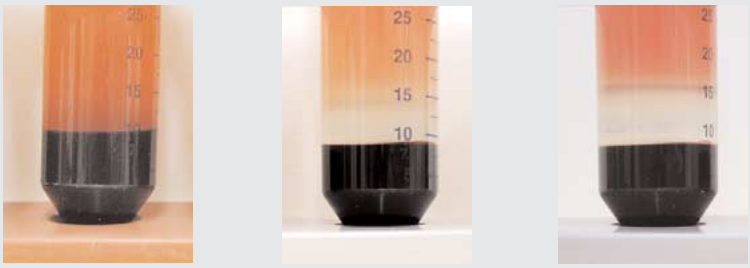

Fig. 1a Fig. 1b Fig. 1c

Fig. 1: Comparison of the gradient formation: Centrifuge 5702 with brake switched on (a), centrifuge 5702 with soft function switched on, variant I (b), centrifuge 5810 as a comparison centrifuge (c)

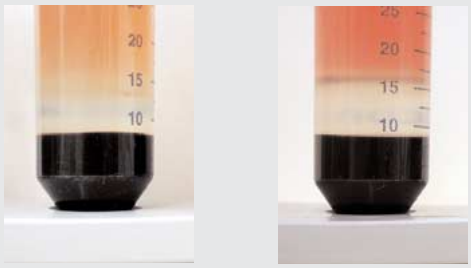

Fig. 2a Fig. 2b

Fig. 2: Comparison of the gradient formation: Centrifuge 5702 with soft function switched on, variant I (a), centrifuge 5702 with soft function switched on, variant II (b)

A significant improvement in the centrifugation results was achieved by the use of a centrifuge with a new software program (variant II). This variant enables both smooth, slow acceleration and smooth, electronically controlled braking. The comparison shown in Fig. 2 reveals clearly that the ring of mononuclear cells is considerably easier to locate in centrifuge 5702 with this software variant (Fig. 2b) than in the centrifuge with the first software variant (Fig. 2a). This results in both a higher cell yield (cf. Table 1) and in a reduction of undesirable cells (e.g. thrombocytes), as shown by means of a stray light analysis (cf. material and methods, results not shown).
The blood used for the experiments described above originated from one donor so that comparability of the results is guaranteed.

Table 1: Description and results of experiments for determining the optimal soft function

Centrifuge	Centrifuge 5702 Software I	Centrifuge 5702 Software I	Centrifuge 5702 Software II	Centrifuge 5810 (comparison model)
Program for Ficoll gradients				
RPM; RCF	1600 rpm; 440 x g	1600 rpm; 440 x g	1600 rpm; 440 x g	1700 rpm; 440 x g
Time (total)	30 min.	30 min.	30 min.	30 min.
Brake, acceleration	Max.	*Soft function*	*Soft function*	Min. (ramp 0/0)
Washing program				
RPM; RCF	30 min.	1600 rpm; 400 x g	1600 rpm; 440 x g	1600 rpm; 440 x g
Zeit (gesamt)	max.	30 min	30 min	30 min
Bremse, Beschleunigung	n.d.	max.	max.	ramp 9/9
Menge MNC	n.d.	152 million	168 million	172 million

n.d.: not detectable

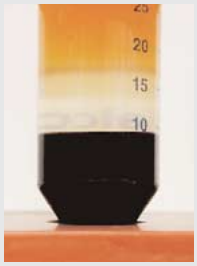

Fig. 3a

Fig. 3b

Fig. 3: Comparison of the gradient formation: Centrifuge 5702 with soft function switched on, variant II (a); Centrifuge 5702 with soft function switched on, variant III (b)

In a third step the software was optimized so that the runtime was as short as possible. Fig. 3 shows a comparison of software variants II and III. The formation of the Ficoll gradients is comparably good in both cases, with variant III allowing considerably shorter braking times (approx. 60 sec. in comparison with approx. 180 sec. for variant II). In this way it was possible to develop a soft function both for acceleration and for braking that allows mononuclear cells to be cleaned quickly with good reproducible results using a Ficoll gradient.

Summary

In this application cooperation between academic research and industrial development has enabled the software for a centrifuge for the clinical routine lab to be optimized. Various versions of the Centrifuge 5702 were investigated for the isolation of human mononuclear cells (MNC) from whole blood by means of density gradient centrifugation. It was evident that, although the first software was suitable for the method, it was possible to further improve the result by optimizing the control software. Thus, Centrifuge 5702 is a laboratory centrifuge that can be used in the clinical routine lab for practically every application. The optimized software variant has naturally been implemented in the other centrifuges in this family, for example in the cooled version, Centrifuge 5702 R, and in the latest centrifuge to join this family, the temperature controlled Centrifuge 5702 RH, that can both heat and cool samples with very precise temperature control (+/- 0.5 °C at 4 °C and 37 °C).

References

[1] Böyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood *Scand.J.Lab.Clin.Invest* 21:77-89.

Ordering information

Article	International Order No.	Brinkmann Order no.
Centrifuge 5702, without rotor	5702 000.019	022 62 620 1
Centrifuge 5702 R, without rotor	5703 000.012	022 62 620 5
Centrifuge 5702 RH, without rotor	5704 000.016	022 62 621 5
Swing-bucket rotor A-4-38,		
incl. 4 à 85 ml round buckets	5702 720.003	022 63 904 8
Adapter for 50 ml Falcon tubes, set of 2	5702 734.004	022 63 922 6
Centrifuge 5810, without rotor		
Swing-bucket rotor A-4-44, incl.	5810 000.017	022 62 350 8
4 rectangular buckets à 100 ml	5804 709.004	022 63 740 1
Adapter for 50 ml Falcon vessels, set of 2	5804 758.005	02263766-5