Research article

EFFECT OF RESTING TIME ON PERIPHERAL BLOOD MONONUCLEAR CELL YIELDS

Inna Narayani[1*], Rasmaya Niruri[1], Nyoman Mantik Astawa[2]

[1] *Faculty of Mathematics and Natural Sciences Udayana University*
[2] *Faculty of Veterinary Sciences Udayana University*
** Corresponding author : in86narayani@yahoo.com*

ABSTRACT

Cryopreservation of PBMC (peripheral blood mononuclear cells) was done to preserve and analyze the number of PBMC derived from blood samples which come in at different time. The batch analysis was performed at the same time in order to reduce variations in results.. The analysis on the cells numbers carried out after 1, 3, 6, 12, and 24 hours. Heparinized whole blood was collected from healthy subject by venipuncture, and stored at room temperature. Blood is processed by centrifugation in Ficoll density gradient following the established method of Balai Besar Veteriner Denpasar. Buffy coat layer was collected and washed twice with HBSS (Hank's balanced salt solution) and was counted in Turk's solution. The cells were then dissolved in 1 ml of cold freezing medium containing 10% DMSO and 50% FBS (fetal bovine serum) and stored overnight at -80°C before storage in liquid nitrogen vessel for few weeks. The samples rapidly thawed in a water bath at 37°C and washed twice with PBS (phosphate buffered saline). The cells were stored in 4°C PBS and counted in Turk's solution after 1, 3, 6, 12, and 24 hours. The results obtained were varied with a declining trend.

Keywords: buffy coat, cell count, , PBMC, resting time

INTRODUCTION

PBMC are live cells which easily damage, some of which start dying immediately after isolation from whole blood. The PBMC has to be analyzed immediately following isolation at the site where they were obtained. The blood requires to be processed and the cells tested one sample at a time as the patient become available, irrespective of the time of the day or week (Olson *et al*., 2011) .When the analysis are performed at different laboratory or hospital, transport and storage of clinical specimens become important variables that may affect PBMC viability and function. (Bull, M. *et al*., 2007, Olson *et al*., and 2011Kutscher, S. *et al*., 2013) The effect of resting time during storage and transport of peripheral blood on quantity represents the focus of this study.

MATERIAL AND METHODS

The study was approved by Sanglah Hospital – Faculty of Medicine, Udayana University ethics committee. The study was conducted at Balai BesarVeteriner (BBVet) Denpasar Bali on period of June – August, 2015.

PBMC processing method

Blood Collection

Venous blood samples were collected from one healthy volunteer at Faculty of Medicine Udayana University by venipuncture in sodium-heparin tubes. The tubes were kept at room temperature and transported

immediately to Balai Besar Veteriner laboratory for separation of PBMCs.

Blood Processing

Blood was processed by centrifugation in Ficoll density (3000 rpm or 1.400 *g*) for 5 minutes in refrigerated swing rotor centrifuge. Buffy coat layer was collected and washed twice with HBSS (Hank's balanced salt solution). The cells were counted under the microscope in Turk's solution and divided into 3 vials for group A, B, C.

Cryopreservation

The cells were then put in cryovials and dissolved in 1 ml of cold freezing medium containing 10% DMSO (dimethylsulphoxide) and 50% FBS (fetal bovine serum) and stored overnight at -80°C. The next day the cells were stored in liquid nitrogen vessel for few weeks.

Thawing

As the samples take out from liquid nitrogen, the cells rapidly thawed in a water bath at 37°C and immediately washed twice with warmed PBS (phosphate buffered saline). The cells were stored in 4°C PBS counted in Turk's solution after 1, 3, 6, 12, and 24 hours.

RESULTS AND DISCUSSION

The numbers of cell after cryopreserved and thawing were counted, the data was shown on Tabel 1.

Tabel. 1 . Number of Cells After Thawing at Different Resting Time

	Resting Time (hour)					
	0	**1**	**3**	**6**	**12**	**24**
Group	**Mean Value Cell Numbers (x 10^3 cells /µl)**					
A	904	884	945	920	720	535
B	1705	1270	1490	1004	620	515
C	1160	1381	1280	910	910	540

There were a decrease of cell numbers after one hour resting at 4°C in PBS followed by a slightly increase in number after 3 hr. As the resting time continued to 6, 12 and 24 hr, the cells tend to decrease. Under ideal conditions, the PBMC can be tested immediately following isolation at the site where they were obtained (CPTP, 2015). Many times, however, this is unfeasible for large multi-center clinical studies (Wunch, M., *et. al*., 2015). When the analysis is performed at multiple laboratories, transport and storage of clinical specimens become important variables that may affect lymphocyte viability and function ing blood and tissue specimens. The effect of temperature during storage and shipment of peripheral blood on subsequent processing, recovery, and function of

lymphocytes are important. (Olson *et al*., 2011). Incubation of PBMC at room temperature for 1 hour did not affect the percentage of apoptotic cells. However, incubation at 4 degrees C for 24 hours resulted in the low percentage of apoptotic cells (Bergman,M. *et al*. 1985).

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