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Journal Name:	Asian Journal of Research in Biochemistry
Manuscript Number:	Ms_AJRB_44599
Title of the Manuscript:	SEPARATION AND OPTIMIZATION OF A SUCROSE DENSITY GRADIENT CENTRIFUGATION PROTOCOL FOR ISOLATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC)
Type of the Article	Original Research Article

General guideline for Peer Review process:

This journal's peer review policy states that <u>NO</u> manuscript should be rejected only on the basis of '<u>lack of Novelty'</u>, provided the manuscript is scientifically robust and technically sound. To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline)

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PART 1: Review Comments

Compulsory REVISION comments The use of a sucrose density gradient centrifugation for isolation of PBMCs is a promising protocol however this manuscript presents some critical points that should be reviewed. Introduction should be reviewed in basis of a more recent bibliography, focusing on atual PBMc uses, and methods used for they obtention. In the first sentence: "Blood is made up of plasma and other types of cells including PBMCs " the PBMC acronym must be defined In materials and methods section authors should inform:	
The use of a sucrose density gradient centrifugation for isolation of PBMCs is a promising protocol however this manuscript presents some critical points that should be reviewed. Introduction should be reviewed in basis of a more recent bibliography, focusing on atual PBMc uses, and methods used for they obtention. In the first sentence: "Blood is made up of plasma and other types of cells including PBMCs "the PBMC acronym must be defined"	
focusing on atual PBMc uses, and methods used for they obtention. In the first sentence: "Blood is made up of plasma and other types of cells including PBMCs " the PBMC acronym must be defined	
in materials section,	
 what kind of centrifuge tubes were used. 	
In methods setction - change the sub head title "Method" to "methods". - Inform about how many blood donations, from how many persons were used. - Centrifugation force should be mentioned in g force and not in rpm.	
The RBC acronym must be defined. Inform about how many time between blood collection and blood	
preparation, and how it was preserved until analysis	
 Clarify about the purpose of lymphocyte culture? - It was to test the functionality? - How? Clarify how the use of the hematology analyzer serves to test cell viability. Also it is important to inform what kind of hematology analyzer was used. Normally cell viability is tested with "Trypan blue exclusion test" In my opinion it is important, prior to use this protocol, as well as prior to its publication, to compare results with other PBMc separation protocols and test parameters as PBMc recovery per mililiter of whole blood. 	
Results should be presented in a more consistent manner, and not only as a legend of a figure. Authors should present consistent results on: -number of cells recovered -purity of PBMc isolate -cells viability -cells functionality	
Discussion After reviewing materials and methods and results section, discussion should be adressed properly.	
In figure 4 legend should exactly explain what is seen in the image	
References Authors should perform a new search for more recent references in order to ameliorate the manuscript	
Minor REVISION comments	

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Optional/General comments	

As per the guideline of editorial office we have followed VANCOUVER reference style for our paper.

Kindly see the following link:

http://sciencedomain.org/archives/20

PART 2:

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Are there ethical issues in this manuscript?	(If yes, Kindly please write down the ethical issues here in details) There is a lack of information about the donnors, if they were volunteers and gave consent to the use of blood	

Reviewer Details:

Name:	Ana Cristina Silvestre Ferreira
Department, University & Country	Universidade de Trás-os-Montes e Alto Douro Portugal

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