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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 **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', 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:

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PART 1: Review Comments

	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)
Compulsory REVISION comments	<p>Please see my comments for the manuscript. Gradient centrifugation is very old techniques for separation of things in a solution.</p> <p>Comments</p> <ol style="list-style-type: none"> Details about sucrose can be added in the introduction part instead of mentioned in Materials and method. In method section: sodium heparin vacutainer was used for blood collection whereas in abstract it was mentioned that citrated blood used for this study. Which one is correct? What is the proportion of sucrose solution added with 4 ml of diluted blood? Why the author used cooling centrifuge (4°C) for centrifugation? Will it room Temperature (27°C) affect the gradient centrifugation process? What is the necessity of lymphocyte culture in this study? For microscopy studies, PBMC can be observed after the isolation step itself!!!!? . Lymphocyte culture method is not in detail. Cells cultured in petri dish/culture flask/tubes!!?. In method section details on Hematology analyzer should be added. Results are not provided in a detailed manner...ie. Supporting data of haemogram should be added. Authors are suggested to add Comparative haemogram analyses of PBMC derived from Ficoll and 40% sucrose gradient method. Fig.2. The final volume of the sample mixture (sucrose+ diluted blood) are not equal in all the tubes? Rewrite the figure legend. .. Fig.4. Microscopic images are doesn't meet the standard, poor in quality. Replace with clear image. Details need to add for Fig 4A. and Fig.4B. In the discussion section authors mentioned, PBMC derived from 40% sucrose centrifugation protocol can be stored for a long time Author's monitored the stability & viability of PBMC in different day interval? 	<p>Accepted and corrected</p> <p>corrected</p> <p>corrected</p> <p>the cell will be more stable in lower temperature</p> <p>To conduct cytotoxicity studies using lymphocyte it will be usefull</p> <p>Corrected</p> <p>Corrected</p> <p>We have counted using older version of hematology analyser in that we didn't got graph</p> <p>We taken ficol results from literature surve since we don't have fun ding for this work ficol is expansive so we used sucrose for the study</p> <p>The volume added is 1;1 proportion but based on the sedimentation rate of hig weight moleculaes the volume will increase in different concentrations Replaced</p> <p>Most of the cells stable in 4 degree so we can store it for shorter duration</p>



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	The standard of English needs to improve throughout this manuscript.	
Minor REVISION comments		
Optional/General comments		

PART 2:

	Reviewer's comment	Author's comment <i>(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)</i>
Are there ethical issues in this manuscript?	<i>(If yes, Kindly please write down the ethical issues here in details)</i>	Ethics clearance taken from institute