



# ASSESSMENT OF NEW PBL ISOLATION METHOD TO OBTAIN A LYMPHOCYTE PREP FREE OF CONTAMINATING CELLS

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## AIM:

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The aim of our study is to modify the current procedure to obtain a cleaner cell preparation.

## INTRODUCTION:

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Background: The current lymphocyte isolation method used in our laboratory results in a preparation that is contaminated with red blood cells in hemolytic samples. Additional steps are required to clean the preparation which prolongs the process for performing stat crossmatches. We are seeking a new technique that will help reduce red blood cell contamination.

## MATERIALS & METHODS:

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Peripheral blood lymphocytes (PBL) from deceased donors or patients were divided into equal volumes. Using the current method, the blood was diluted 1:2 and overlaid with Histopaque in 50 ml Leucosep tubes (Greiner). The second half of PBL was diluted 1:4 and overlaid with Histopaque in leucosep tubes. The sample tubes were centrifuged following the guidelines. Then, resulting cells were counted using Sysmex cell counter to determine cell purity, red blood cells contamination and polynuclear cell contamination.

## RESULTS:

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Comparison of Leucosep dilutions using 15ml Leucosep tubes:

SAMPLE:	1	2	3	4	5
LYMPH :	13.7	25.4	15.4	10.2	27
LYMPH %:	48.4%	70.1%	63.8%	57.1%	67.5%
POLYS %:	51.6%	29.9%	36.2%	42.9%	32.5%
BLOODY	No	No	No	Yes	Yes

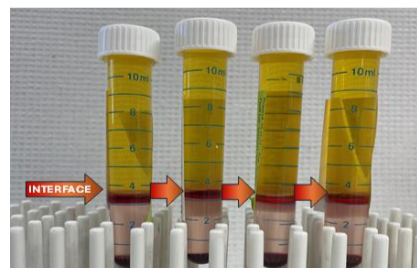
1	2	3	4	5
11.1	18.8	6.8	3.2	26.8
49.3%	67.6%	61.0%	46.3%	67.9%
50.7%	32.4%	39.0%	53.7%	32.1%
No	No	No	Yes	Yes

### Interface

Current Method (1:2)



Proposed Method (1:4)



### Pellet

Current Method (1:2)



Proposed Method (1:4)



## CONCLUSION AND ANALYSIS:

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The 1:4 dilution cell preparation does not improve lymphocyte yield, or reduce contaminating cells