

## A PRELIMINARY STUDY OF EFFECTS OF DELAYED SEPARATION AND CRYOPRESERVATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS ON VIABILITY AND RECOVERY

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### ABSTRACT

**Background:** The cryopreserved of viable peripheral blood mononuclear cells (PBMC) for later use is essential for virological and immunological studies especially the assays used are technically complex and long processing.

**Objectives:** To examine the effects of delayed separation and cryopreservation of PBMC on viability and recovery results.

**Materials and Methods:** Acid citrate dextrose (ACD) blood was collected from 6 healthy blood donors and PBMC was isolated by Ficoll-Hypaque separation after holding the ACD blood overnight (1 day), 2 and 3 days at 4°C. Fresh PBMC was counted and viability was estimated by trypan blue dye exclusion. PBMC of each day was cryopreserved and thawed. Cell counting and viability of cryopreserved PBMC were performed to compare with fresh PBMC.

**Results:** Percentages of viability of fresh PBMC of 1, 2 and 3 days storing the ACD blood at 4°C were 100% whereas viability of cryopreserved PBMC of 1, 2 and 3 days storing the ACD blood at 4°C were decreased to 96.29%, 93.39 % and 92.73%, respectively. There was significant differences in percentage of viability between fresh and cryopreserved PBMC of 1, 2 and 3 days storing the ACD blood at 4°C ( $p = 0.028$ ,  $p = 0.027$ ,  $p = 0.028$ , respectively). However, there was no significant difference between viability of fresh PBMC of 1, 2 and 3 days storing the ACD blood at 4°C. Percent of recovery of cryopreserved PBMC of 1, 2 and 3 days storing the ACD blood at 4°C were at least 80 %.

**Conclusions:** The preliminary data indicated that fresh PBMC can be separated from ACD blood stored at 4°C at least 3 days with 100 % viability but viability and recovery of cryopreserved PBMC were decreased over time..

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