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STANDARDIZATION OF THE FICOLL GRADIENT TECHNIQUE FOR THE ISOLATION OF MONONUCLEAR CELLS FROM PERIPHERAL BLOOD

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Objective: The objective of this study was to standardize the Ficoll gradient technique for the circulating hematopoietic stem cell (HSC) isolation for the assembly of the peripheral blood mononuclear cell (PBMC) biorepository of breast cancer (BC) patients attended in the Clinical Oncology Service of Instituto Mário Penna. **Methods:** The study protocol was approved by the Ethics Committee of Instituto Mário Penna (CAEE 82703418.8.0000.5121). In recommended protocols, 15 mL of blood was used. At first, we adapted this volume due to the limited amounts of samples for research available. Blood was collected in a 9-mL sodium heparin tube. The experiments were performed in 50-mL conical tubes, but with reduced blood volume, and no PBMC ring was formed. It was necessary to change to 15 mL conical tubes. Finally, the remaining red blood cells were lysed with ammonium chloride. However, with the reduced volume, this solution lysed the PBMC too. Then, we decided to remove this step from the protocol. **Results:** We obtained 8.06×10^6 cells/mm³ with 80% viability. Data were confirmed by a Neubauer camera and an automatic cell counter. The HSCs were labeled with antibodies against CD34 and CD133 by flow cytometry. **Conclusion:** The characterization of HSCs is important to link tumor-associated HSCs with malignant and immunosuppressive phenotypes. Studies are in progress with this standardization, and they will permit us to perform the HSC characterization of BC patients with a better knowledge of tumor microenvironment.

Keywords: Mononuclear cells. Hematopoietic stem cells. Ficoll.