Evidence of Paraprotein Interference with the CD138+ Enrichment Technique
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BACKGROUND

Multiple myeloma (MM), also known as Plasma Cell Myeloma, is a disease characterized by the uncontrolled proliferation of CD138+ plasma cells (PCs) which can lead to the increased production of abnormal immunoglobulins or paraproteins in serum and/or urine. Hyperproteinaemia is a key indication of multiple myeloma, when the total plasma protein exceeds 8.5 g/dL. If the paraprotein immunoglobulin levels alone exceed 6 g/dL, hyper-viscosity of the plasma occurs causing rouleaux erythrocytes and other further complications. In order to detect the most frequent genomic abnormalities associated with MM, FISH is utilized. However, FISH can be limited by the number of plasma cells in the sample. Therefore a plasma cell enrichment technique for the CD138+ marker is utilized.

MATERIALS and METHODS

CD138+ target cells are labeled with dextran coated magnetic particles using bispecific Tetrameric Antibody Complexes (TAC’s). These complexes recognize both dextran and target cell surface antigen. Magnetically labelled cells are then separated from unlabelled cells using an EasySep magnet. Unwanted cells are poured off leaving the CD138+ labelled cells in the tube (Fig. 2). A direct harvest is then performed on purified cells to prepare for FISH analysis.

RESULTS

The EasySep™ Human Whole Blood and Bone Marrow CD138+ immunomagnetic enrichment technique was performed on 84 bone marrow and leukemic blood samples with an indication of MM or monoclonal gammapathy. The % PCs by pathology ranged from 5% of a 10% hypocellular bone marrow to 95% of a 98% hypercellular bone marrow. A sufficient amount of PC’s was collected on all but 2 samples (2.4%) to perform a 7 probe MM FISH panel. These 2 failed samples had approximately 90% of a 40% normocellular bone marrow and 80% of a 50% mildly hypercellular bone marrow PC’s by pathology. Both samples indicated hyperproteinaemia with total proteins of 11.8 and 9.5 g/dL. Further investigation showed all samples prior to these 2 samples using our current enrichment method had total proteins ≥8.5 g/dL. Suspecting evidence of protein interference in the enrichment technique, we diluted a portion of 2 samples with a total protein of 9.3 g/dL (90% of a 95% and >90% of an 85% PC’s, respectively) before proceeding with enrichment and successfully retrieved adequate PC’s on both samples. Additionally, we conducted a side by side enrichment technique on a sample with a total protein of 9.0 g/dL and a PC concentration of 90% of a 70% hypercellular bone marrow. Both diluted and undiluted samples yielded PC’s; however, the diluted sample pellet was visibly larger and resulted in a cleaner FISH analysis, while the undiluted and direct cells had poor morphology and were difficult to analyze. Additionally, we are exploring other markers to aid in sample preparation if the protein level is not available or recent.

CONCLUSIONS

We have demonstrated evidence of endogenous protein interference with the immunomagnetic CD138+ enrichment technique due to disruption of the antibody binding. In order to alleviate the interference, our laboratory implemented a dilution table based on the total protein of all MM samples (Table 1). To date, we have diluted 23% of samples before proceeding with enrichment and all yielded adequate PC’s for FISH analysis. We recommend that labs using this specific procedure check the protein level of their MM samples before proceeding with the enrichment. If the levels are high, a dilution is highly recommended to ensure adequate capture of PCs. If total protein is not available, Serum Protein Electrophoresis (SPEP) and Immunofixation Electrophoresis (IFE) are frequent finding in MM patients and an indication of the presence of excess M-proteins or paraproteins.

REFERENCE

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