Evidence of Paraprotein Interference with the CD138+ Enrichment Technique

Connie Monroe, MT(ASCP), SC^{CM}, CG^{CM} and Holli M. Drendel, Ph.D., FACMG
Fullerton Genetics Laboratory
Mission Health, Asheville, NC

BACKGROUND

Multiple myeloma (MM), also known as Plasma Cell Myeloma, is a disease characterized by the uncontrolled proliferation of CD138+ plasma cells (PC's) which can lead to the increased production of abnormal immunoglobulins or paraproteins in serum and/or urine. Hyperproteinemia 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.

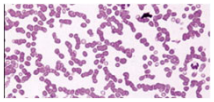

Figure 1: Rouleaux formation

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 gammopathy. The % PC's 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 hyperproteinemia 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.

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 unlabeled 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.

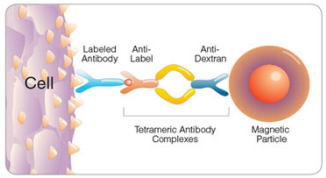

Figure 2: Magnetic CD138+ cell separation from Stemcell Technologies. EasySep™ Kit

RESULTS

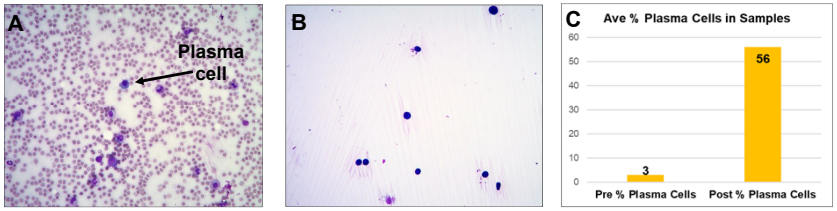

Figure 3: Validation of PC enrichment: A) 200X Wright stained BM sample showing pre-enrichment with **3%** PC's per 100 cell differential. B) 200X Wright stained post enrichment BM sample with **97%** PC's per 100 cell differential. C) Average % PC's in pre and post enrichment of validation samples.

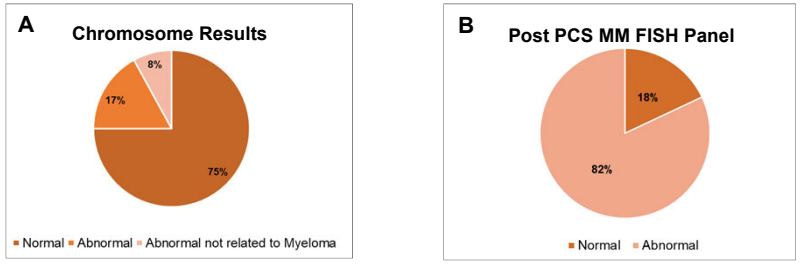

Figure 4: Percent abnormal of cultured MM samples vs enriched MM samples: A) % of karyotypes with abnormalities related to MM, unrelated abnormalities and normals. B) % of PCS FISH studies with abnormalities. 2 (5%) PCS failures; one with 2% PC's by pathology and a normal MM FISH panel and another with no total protein available and both abnormal MM FISH panel and chromosomes.

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 preceding with the enrichment. If the levels are high, a dilution is highly recommended to ensure adequate capture of PC's. If total protein is not available, Serum Protein Electrophoresis (SPEP) and Immunofixation Electrophoresis (IFE) (Figure 5) identify the presence of an M-spike in the beta or gamma region and calculates the M-protein concentration. >3.0 g/dl indicates excessive M-proteins or paraproteins present. Beta-2 microglobulin is used as a prognostic marker to stage MM disease, elevated values (>3 mg/L) correlate with increased plasma cell activity and could also indicate an elevated total protein.

CONCLUSIONS

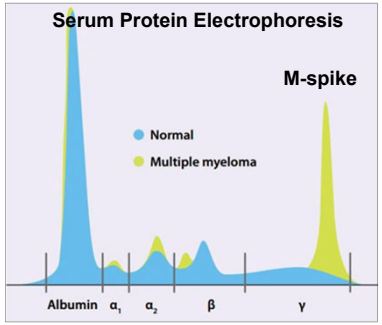

Figure 5: M-spike in the beta or gamma region on Serum Protein Electrophoresis (SPEP) or Immunofixation Electrophoresis (IFE) is a frequent finding in MM patients and an indication of the presence of excess M-proteins or paraproteins.

Table 1: Plasma Cell Separation Dilution Chart

Total Protein	BM volume in mL	PBS w/ 2%FBS- mL	CD138+ Cocktail and Spheres
≤ 8.5 g/dl	0.5	1.5	50 µl
	0.75	1.25	
	1.0	1.0	
8.6-11.0 g/dl	0.5	1.5	50 µl
	0.75	2.25	75 µl
	1.0	2.0	75 µl
>11.0 g/dl	0.5	1.5	50 µl
	0.75	2.25	75 µl
	1.0	3.0	100 µl

REFERENCES

1. Stemcell Technologies website and EasySep Human Whole Blood and Bone Marrow CD138+ package insert
2. Clinical Chemistry, A Laboratory Perspective, Arneson and Brickell
3. Science Direct, 2017 (Figure 1 image)
4. Best tests, July 2011 (Figure 5 image)
5. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 2017
6. International Myeloma Working Group

ACKNOWLEDGEMENTS

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