



# Saving valuable hands-on time – automated CD138<sup>+</sup> plasma cell enrichment

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## Background

Multiple myeloma (MM) is a plasma cell (PC) neoplasm in which the PC surface antigen CD138 (syndecan-1) is highly expressed. Abnormalities in MM PCs can be detected by FISH analysis. We have previously observed higher abnormality rates when FISH analysis was performed on uncultured, enriched CD138<sup>+</sup> cells (50.8%) in comparison to routinely cultured bone marrow cells (7.4%,  $P < 0.001$ ). In our laboratory, CD138<sup>+</sup> cell enrichment has subsequently become standard practice prior to FISH and chromosome microarray analysis of MM samples. As manual PC enrichment with different commercial kits is time consuming, we validated and implemented the autoMACS<sup>®</sup> Pro Separator for automated enrichment of CD138<sup>+</sup> PCs.

## Materials and methods

### Enrichment of CD138<sup>+</sup> plasma cells

We enriched CD138<sup>+</sup> PCs from 12 bone marrow samples using the MACSprep<sup>™</sup> Multiple Myeloma CD138 MicroBeads, human (Miltenyi Biotec, 130-111-744). Separation was performed following the manufacturer's instructions for either manual separation or automated separation with the autoMACS Pro Separator (Miltenyi Biotec).

### Bone marrow preparation

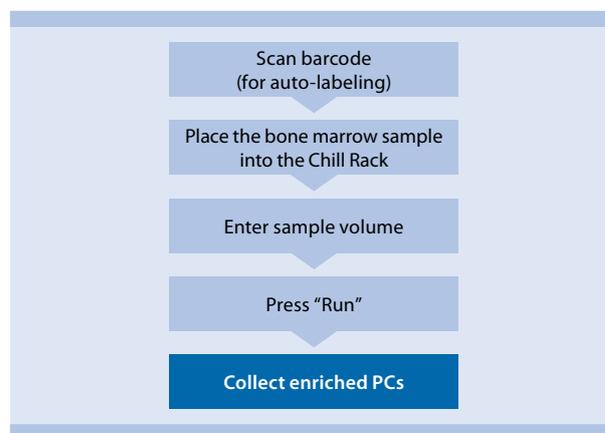
Bone marrow samples were prepared by removing the bone fragments with filters and washing with autoMACS Running Buffer. After centrifugation, the cell pellet was resuspended in buffer for manual or automated cell separation.

### Manual cell separation

The bone marrow cell suspension was labeled with MACSprep Multiple Myeloma Microbeads and applied onto a MACS<sup>®</sup> Whole Blood Column placed within a magnetic field. After washing the column with buffer, it was removed from the magnet and the labeled CD138<sup>+</sup> cells were eluted with Whole Blood Column Elution Buffer.

### Automated cell separation

In order to prepare the automated cell separation process (Fig. 1), the QR code on the MACSprep Multiple Myeloma MicroBeads reagent was scanned using the barcode reader on the autoMACS Pro Separator. The bone marrow cell suspension and MicroBeads were then placed into the MACS MiniSampler. Subsequent autolabeling, incubation, and cell separation was performed fully automatically with the Posselwb program. Finally, the separated fractions were collected from the instrument.



**Figure 1: Automated cell separation with the autoMACS Pro Separator.**

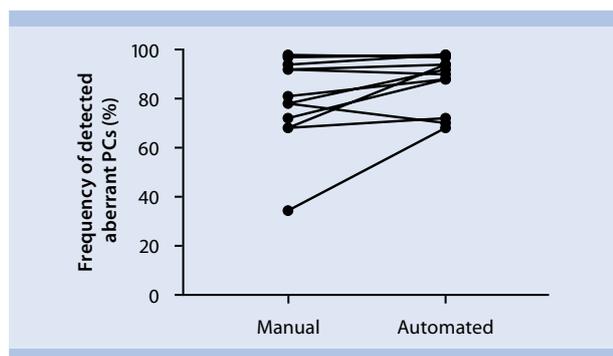
### FISH analysis

FISH analysis was performed using dual-fusion dual-color probes for IGH/CCND1, IGH/FGFR3, IGH/MAF, as well as IGH break-apart, TP53/D17Z1 and / or KKS1B/CDKN2C locus-specific probes (Carl Zeiss). Abnormal cells detected by FISH analysis after manual vs. automated separation were compared using McNemar's test for paired samples with a significance level  $\alpha = 0.05$ .

## Results

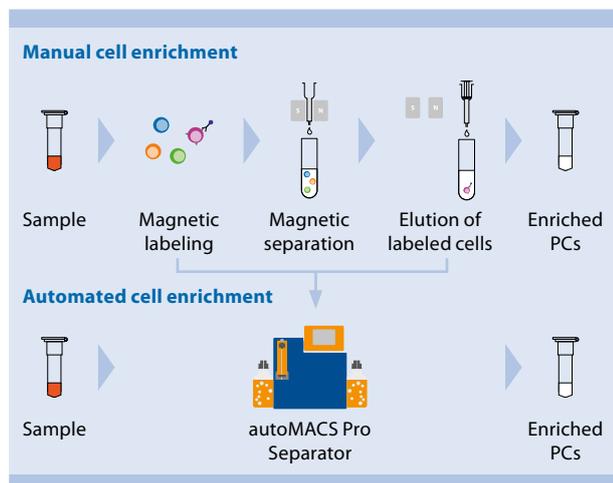
In order to validate the performance of automated PC enrichment, we compared the frequency of aberrant CD138<sup>+</sup> PCs detected by FISH analysis in each MM bone marrow sample enriched in parallel, either manually or using the autoMACS Pro Separator. The frequency of

aberrant PCs is comparable between both, manual and automated methods in two-thirds of the samples (8/12; difference <8%). However, in one-third of the samples, using the autoMACS Pro showed higher frequency of aberrant PCs detected by FISH (4/12; difference >10%) (Fig. 2). The average frequency of aberrant PCs in MM bone marrow samples detected by FISH analysis after automated cell enrichment was 87.4%. The frequency of aberrant cells detected after manual enrichment was slightly but significantly ( $X^2 = 4.6$ ;  $df = 1$ ;  $0.05 < P < 0.025$ ) lower with 79.3%.



**Figure 2: Frequency of aberrant PCs detected in MM bone marrow samples.** The frequency of aberrant plasma cells was analyzed by FISH after parallel manual or automated CD138<sup>+</sup> cell enrichment. Each data point represents one individual bone marrow sample.

As shown in figure 3, automated CD138<sup>+</sup> cell pre-enrichment eliminated time-consuming hands-on steps otherwise performed by the operator, including labeling, separation, and washing. After separation, the enriched PCs could be directly collected for further analysis.



**Figure 3: Manual versus automated pre-enrichment workflows.** Enrichment of PCs directly from whole blood using the autoMACS Pro Separator (lower) saves hands-on time and minimizes the procedure compared to manual cell enrichment (upper).

## Conclusions

The marginally increased frequency of aberrant CD138<sup>+</sup> cells detected by FISH analysis (8.1%) following automated enrichment of PCs compared to manual cell separation can be explained by the small sample size in this experiment. Most notably, the autoMACS<sup>®</sup> Pro Separator reduced the hands-on processing time by around 45 minutes. In conclusion, the paramount benefit of the autoMACS Pro Separator for a busy cytogenetics research laboratory is saving hands-on time for sample processing, or in this case automated enrichment of CD138<sup>+</sup> cells. The instrument can process a minimal sample volume of ~200  $\mu$ L and up to six samples in one batch and is therefore optimal for small and large sample volumes.

Additionally, we demonstrated the robustness of PC enrichment using the autoMACS Pro Separator as shown by the stable detection rate of aberrant PCs. This robustness is especially important as it enables standardized workflows without operational bias for the daily lab routine. The automated cell separation is also easy and safe for handling hazardous samples. Furthermore, the system can be used with a variety of surface marker-specific MicroBeads available for other cell types and might thus be used for other applications, e.g., characterization of B cells (CD19<sup>+</sup> or CD20<sup>+</sup>) for the research of blood-related malignancies.



- Minimal hands-on time with fully automated cell labeling and separation
- True walk-away cell separation with sensor-controlled processes, including startup and housekeeping
- Flexible, easy-to-use benchtop instrument for a multi-user environment



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