# Can Microsampling Devices Coupled with Intact Mass High-Resolution Mass Spectrometry Improve M-Protein Monitoring and Simplify Minimal Residual Disease Assessment in Multiple Myeloma?

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**POSTER NUMBER: M1130-01-02**

## PURPOSE

- Multiple myeloma (MM) is a type of cancer of the bone marrow characterized by an abnormal growth of the number of plasma cells, which produce a monoclonal antibody (M-protein). Patients with MM have a moderate life expectancy and often require multiple lines of chemotherapy for durable control and long-term survival.[1]
- Measurable residual disease (MRD) is an important prognostic factor in MM to evaluate the depth of treatment response which is widely used in clinical trials. MRD is therefore monitored using techniques that measure the number of tumor cells in the bone marrow (BM). Multiparametric Flow Cytometry (MFC), Next Generation Sequencing (NGS), have been developed to provide sensitive MRD assessment, however, BM-MRD, is invasive and may not accurately represent disease burden due to BM site heterogeneity.[2]
- Mass Spectrometry (MS) could offer non-invasive means of assessing the efficacy of the treatment, by measuring the M-Protein in peripheral blood, bypassing the need for BM aspiration.[3]

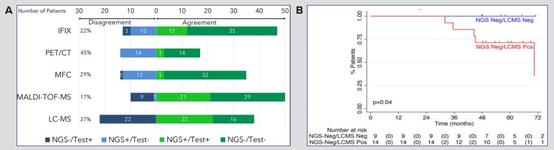



**Figure 1.** A) Dis/agreement between NGS and other MRD assays. B) Progression-free survival (PFS) of all NGS− patients, stratified by LC-MS status.

## OBJECTIVE

The goal of this study is to develop a multi-matrix, patient-centric approach that supports both traditional and self-collection of blood-derived samples using 20 µL Mitra® microsampling devices. Combined with high-resolution mass spectrometry, this workflow enables direct quantification of M-protein. By offering a minimally invasive method, it allows for more frequent and convenient MRD monitoring, potentially enhancing both the accuracy and timeliness of disease tracking.

## METHOD

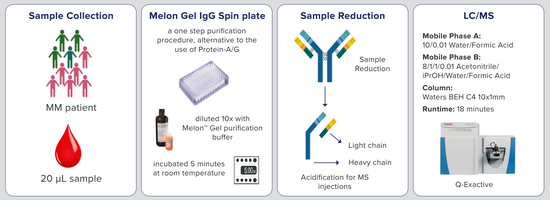
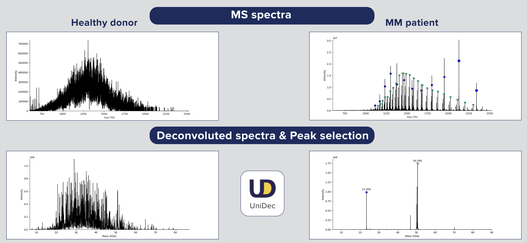



**Figure 2:** Analytical Workflow: deconvolution of MS spectra with UniDec4 software for peak selection and plotting in samples from healthy donors (no dominant species) and MM patients (distinct signal generated by M-Protein).




## RESULTS

### Precision and Accuracy

**Table 1:** Precision and accuracy run.

| M-Protein Calibration Curve | LC Standards in Surrogate Matrix (µg/mL) | | | | | | | | LC Quality Control in Surrogate Matrix (µg/mL) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | STD1 | STD2 | STD3 | STD4 | STD5 | STD6 | STD7 | STD8 | QC-LLOQ | QC-1 | QC-2 | QC-3 |
| | 0.50 | 1.00 | 3.00 | 6.00 | 12.00 | 20.00 | 40.00 | 50.00 | 0.50 | 1.50 | 10.00 | 35.00 |
| Light chain | 0.50 | 0.98 | 2.97 | 5.99 | 12.31 | 23.20 | 37.84 | 44.59 | 0.56 | **1.93** | 9.00 | 32.65 |
| | | | | | | | | | 0.53 | 1.75 | 10.31 | 35.73 |
| | | | | | | | | | 0.40 | 1.70 | 8.43 | 29.41 |
| n | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 |
| Mean | 0.50 | 0.98 | 2.97 | 5.99 | 12.31 | 23.20 | 37.84 | 44.59 | 0.49 | 1.80 | 9.25 | 32.60 |
| SD | | | | | | | | | 0.08 | 0.12 | 0.96 | 3.16 |
| % C.V. | | | | | | | | | 17.1 | 6.6 | 10.4 | 9.7 |
| % bias | 0.9 | -2.2 | -1.0 | -0.1 | 2.6 | 16.0 | -5.4 | -10.8 | -1.1 | 19.8 | -7.5 | -6.9 |

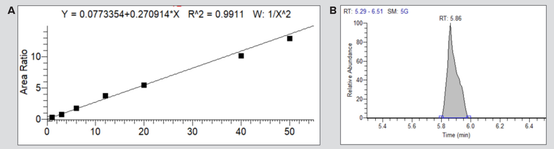



**Figure 3: A)** Standard curve generated by spiking Kappa sFLC spiked into surrogate matrix (PBS-BSA) at different concentrations; **B)** Extracted ion chromatogram for the lower limit of quantitation in surrogate matrix.

### Sensitivity in matrix

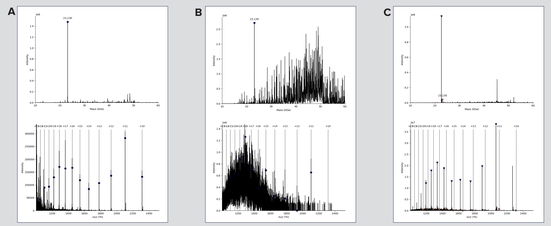



**Figure 4:** Zero-charge Mass Spectra (Top) and Data with Offset isolated species (bottom). **A)** 5 µg of sFLC kappa standard spiked in surrogate matrix. **B)** 50 µg of sFLC kappa standard spiked in a healthy volunteer **C)** 50 µg of sFLC kappa standard spiked in a MM patient with high M-Protein level.

### Robustness and Reproducibility

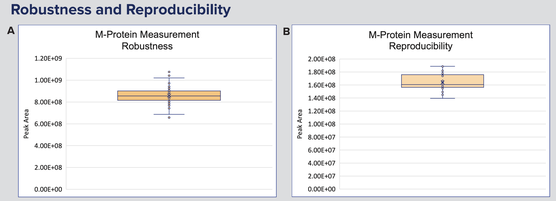



**Figure 5: A)** Method robustness tested on 96 injections of pooled samples extract after purification and reduction; **B)** Method reproducibility tested on 30 independent replicates with up to 5% hemolysis.

### Test of clinical samples

**Table 2:** Assessment of M-Protein (LC only) intensity in plasma and serum over multiple timepoints.

| Timepoint | Plasma Sample | | | Serum Sample | | | |
|---|---|---|---|---|---|---|---|
| | Light Chain Identified | Deconvoluted Mass | Peak Intensity | Light Chain Identified | Deconvoluted Mass | Peak Intensity | % Variation Plasma vs. Serum |
| T1 | YES | 23642 | 4.54E+07 | YES | 23642 | 4.84E+07 | -6.2% |
| T2 | YES | 23642 | 4.18E+07 | YES | 23643 | 4.50E+07 | -7.1% |
| T3 | YES | 23642 | 4.48E+07 | YES | 23643 | 4.42E+07 | 1.4% |
| T4 | YES | 23643 | 4.70E+07 | YES | 23642 | 4.39E+07 | 7.1% |
| T5 | YES | 23642 | 3.86E+07 | YES | 23643 | 4.38E+07 | -11.9% |
| T6 | YES | 23643 | 4.38E+07 | YES | 23642 | 4.18E+07 | 4.8% |
| T7 | YES | 23642 | 4.78E+07 | YES | 23642 | 4.50E+07 | 6.2% |

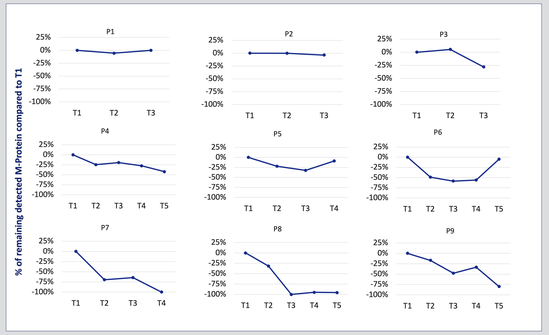



**Figure 6:** M-Protein measurement over multiple timepoints (T1-T5) for nine different MM-patients (P1-P9).

### Development of a patient-centric approach


**Figure 7:** Steps of using Volumetric adsorptive microsampling (VAMS).

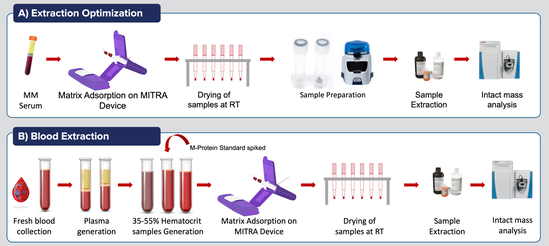



**Figure 8:** Extraction optimization using different homogenization strengths, B) hematocrit level and blood extraction test.

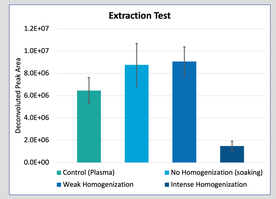



**Figure 9: Mitra extraction results;** Simple soaking and Zirconia offers better sensitivity than simple control. Some impurities remain in the MITRA device, improving the instrument baseline.

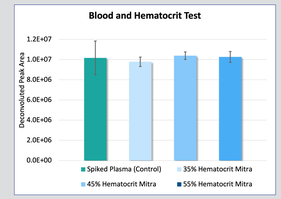



**Figure 10: Blood extraction results;** Average 100% recovery from blood compared to fresh plasma (standard protocol). No Hematocrit effect across the healthy range.

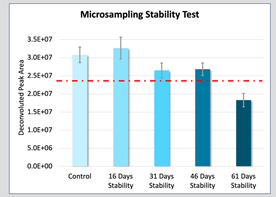



**Figure 11: Stability;** The stability was determined by analyzing aliquots of the medium spike level sample used in repeatability studies after storage at RT for 16, 31, 46 and 61 days

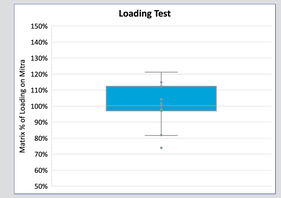



**Figure 12: Mitra Vams Loading Test;** Mitra VAMS devices used for the microsampling stability test were weighed before and after sample loading. The experiment showed consistent reproducibility, with almost the totality of samples falling within ±20% of the nominal volume.

## CONCLUSION

- M-Protein measurement can be achieved with a High-Resolution Mass Spectrometry workflow using just 20 µL of plasma/serum, reducing the need for painful BM biopsy. This method is sensitive, precise, and accurate despite the absence of an internal standard.
- Intact High-Resolution Mass Spectrometry offers the best sensitivity for measuring IgG produced by malignant cells, with a fast assay that processes up to 96 samples in about two hours. It was successfully used to measure M-Protein levels in MM patients, showing over 90% reduction in some cases.
- The method demonstrates excellent robustness and reproducibility, with %CV across replicates below 10%, highlighting the overall solidity of the results from start to finish of the procedure.
- The patient-centric approach for measuring M-protein levels in peripheral blood demonstrated success, with similar or better recovery than plasma. The method is unaffected by red cells (both sample processing and LC/MS), is hematocrit-independent, and is stable for 46 Days.

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