Patient-Centric Measurement of M-Protein Using High-Resolution Intact Mass Spectrometry

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INTRODUCTION

- Multiple myeloma (MM) is a type of cancer of the bone marrow characterized by an anormal 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), and imaging techniques, 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]
- To overcome all these difficulties, 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]
- Derman et al., showed that LC-MS outperforms NGS and MALDI-TOF-MS in predicting PFS. LC-MS-negative patients showed no progression or death, indicating a higher sensitivity compared to the standard techniques. Data also showed that MS was able to detect relapse in earlier timepoints compared to NGS.[3]
- In this study, we present a blood-based method using mass spectrometry for M-Protein detection and their applications of MRD detection in MM patients from assay characterization, to data analysis from plasma/serum samples in a clinical trial.

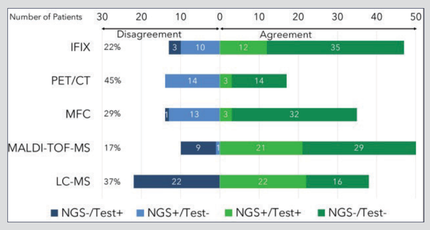
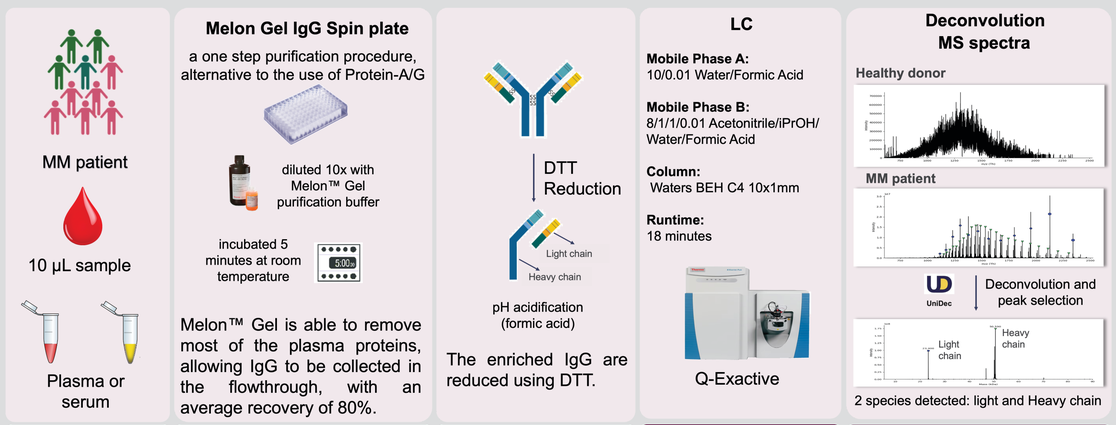


Figure 1. A) Dis/agreement between NGS and other MRD assays. **B**) Progression-free survival (PFS) of all NGS− patients, stratified by LC-MS status.[3]

SAMPLE PROCESSING & DATA ANALYSIS WORKFLOW



PRECISION AND ACCURACY

The final analytical workflows include aliquoting 5µL of mouse or rat plasma, followed by a simple organic solvent denaturation and direct digestion of the samples. Filtration post digestion is added to ensure additional instrument robustness.

Table 1. Precision and accuracy run .

Melon Gel Sample Analysis	*Kappa Light Chain STD in Surrogate Matrix (µg/mL)*								*Kappa Light Chain QC 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

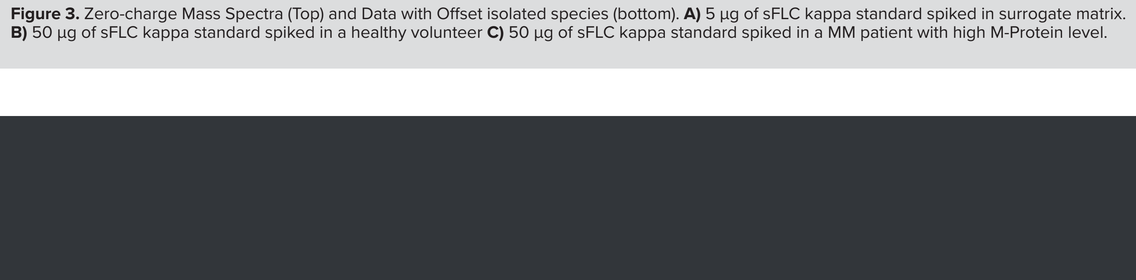


Figure 2. A) Standard curve generated by spiking Kappa sFLC spiked into surrogate matrix (PBS-BSA) at different concentrations; **B)** Extracted ion chromatogram for the lower limit of quantitation.

SENSITIVITY IN MATRIX

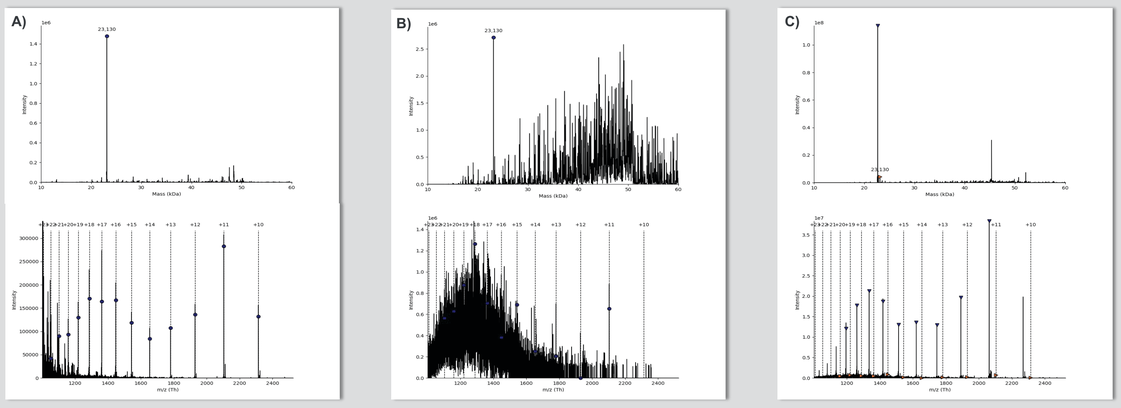


Figure 3. Zero-charge Mass Spectra (Top) and Data with Offset isolated species (bottom). **A)** 5 µg of sFLC kappa standard spiked in surrogate matrix. **B)** 50 µg of sFLC kappa standard spiked in a healthy volunteer **C)** 50 µg of sFLC kappa standard spiked in a MM patient with high M-Protein level.

RESULTS IN CLINICAL SAMPLES

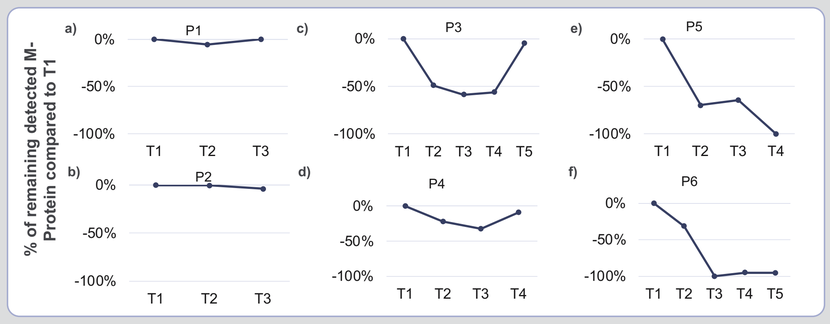
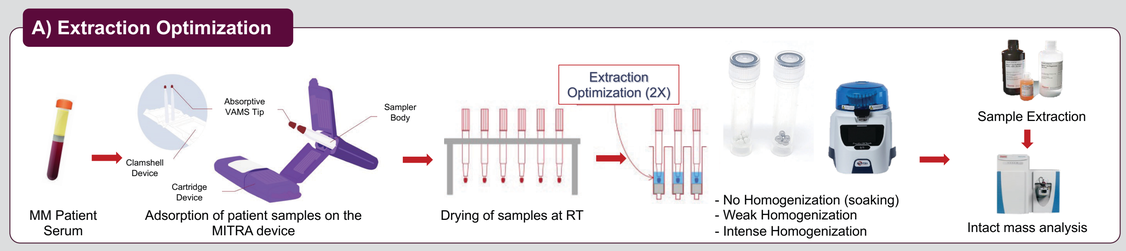


Figure 4. M-Protein measurement over multiple timepoints (T1-T5) for six different MM-patients (P1-P6).

IMPROVING MONITORING, PATIENT-CENTRIC APPROACH

Figure 5. Steps of using Volumetric adsorptive microsampling (VAMS).



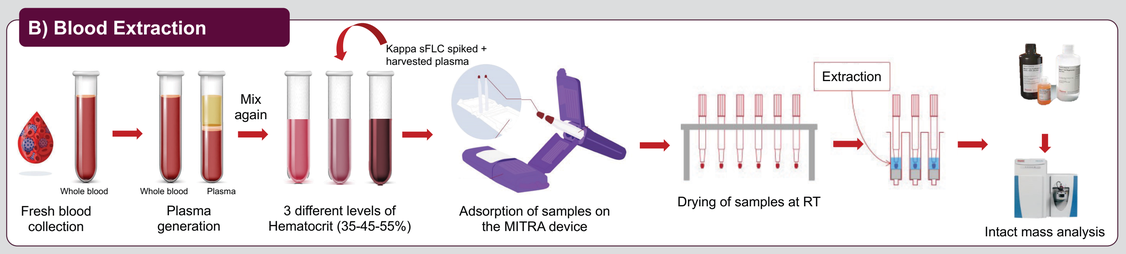


Figure 6. A) Extraction optimization using different homogenization strengths, **B)** hematocrit level and blood extraction test.

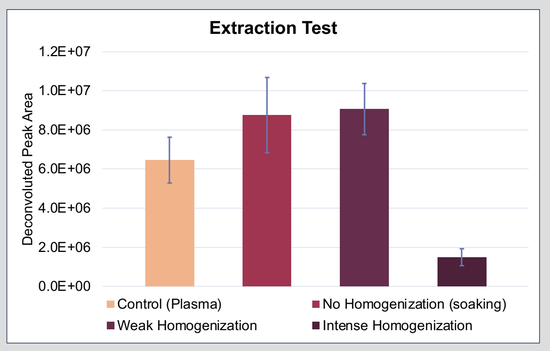


Figure 7. Mitra extraction results; Simple soaking and Zirconia offers better sensitivity than simple control. Some impurities remain in the MITRA device, improving the instrument baseline.

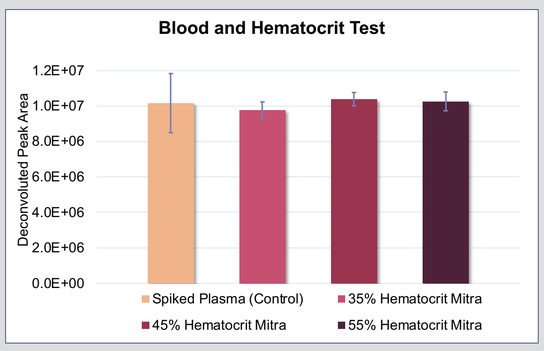


Figure 8. Blood extraction results; Average 100% recovery from blood compared to fresh plasma (standard protocol). No Hematocrit effect across the healthy range

STABILITY AND REPRODUCIBILITY

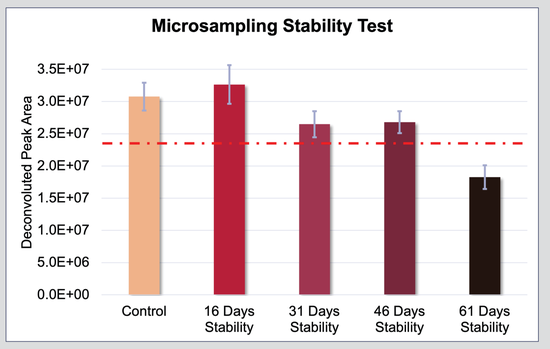


Figure 9. Stability; The stability was determined by analyzing aliquots of the medium spike level sample used in repeatability studies after storage at RT for 16, 31, 46 and 61 days

Figure 10. 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 10 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 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.

REFERENCES

1 MONTEITH, Bethany E.; SANDHU, Irwindeep; LEE, Ann S. Management of multiple myeloma: a review for general practitioners in oncology. Current Oncology, 2023, 30.5: 4382-4401.
2 PERROT, Aurore, et al. Minimal residual disease negativity using deep sequencing is a major prognostic factor in multiple myeloma. Blood, The Journal of the American Society of Hematology, 2018, 132.23: 2456-2464.
3 DERMAN, Benjamin A., STEFKA, Andrew T., JIANG, Ken, et al. Measurable residual disease assessed by mass spectrometry in peripheral blood in multiple myeloma in a phase II trial of carfilzomib, lenalidomide, dexamethasone and autologous stem cell transplantation. Blood Cancer Journal, 2021, vol. 11, no 2, p. 19.
4 MARTY, Michael T., et al. Bayesian deconvolution of mass and ion mobility spectra: from binary interactions to polydisperse ensembles. Analytical chemistry, 2015, 87.8: 4370-4376.

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