#P20

Quantification of sBCMA in Human Plasma using a High-Throughput Mass Spectrometry Workflow for Exploratory, CAP/CLIA or Regulated Studies

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INTRODUCTION

- **Measurement of sBCMA in MM patient plasma**
  - B cell maturation antigen (BCMA) contributes to Multiple Myeloma (MM) pathophysiology
  - BMCA is targeted by various immunotherapies* including CAR T-cells and antibody-drug-conjugates
  - Soluble BCMA (sBCMA) is a promising biomarker for disease prognosis and monitoring and measurements are largely performed using research use only (RUO) commercial ELISA kits
- **ELISA assays for sBCMA measurement may be problematic due to binding of natural ligands and/or therapeutic drug**
- **Potential ELISA limitations may be circumvented by use of mass spectrometry-based assays for precise accurate total sBCMA measurement**

* Also under development: Next gen CAR-T, bispecific T-cell engagers, bispecific molecules, and bi-/tri-specific antibodies

GOALS / OBJECTIVES

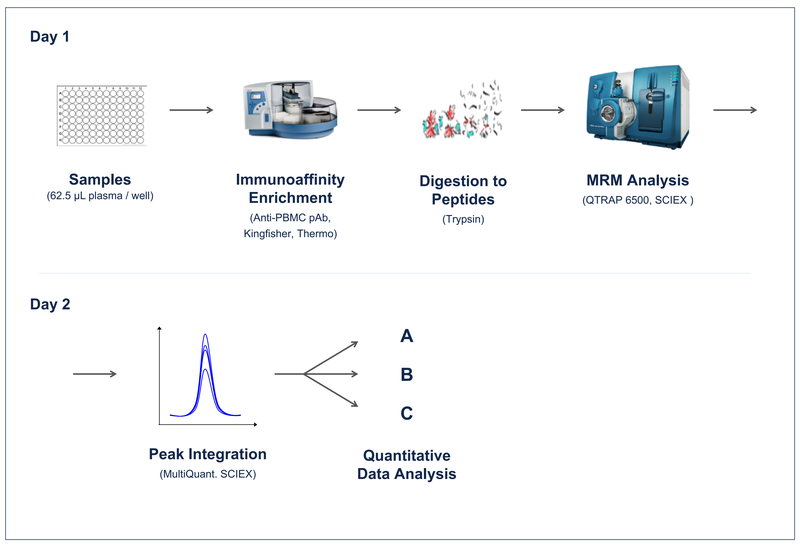
- **To address the need for bioanalytically validated methods for reliable sBCMA measurement in clinical samples**
- **To develop and evaluate suitability of different MRM-based approaches to sBCMA quantitation in clinical plasma samples**

METHODS

SAMPLES

External Curve (n=1):	Surrogate matrix (rat plasma) + sBCMA recombinant protein + SIL peptide (fixed)	STD1 to STD8
QC Samples (n=2):	Authentic matrix (human plasma) + sBCMA recombinant protein + SIL peptide (fixed)	QC0 endo QC2 mid QC3 high
STD0 (n=2):	Surrogate matrix (rat plasma) + SIL peptide (fixed)	
Blanks (n=2):	Rat plasma	

PROCESSING WORKFLOW

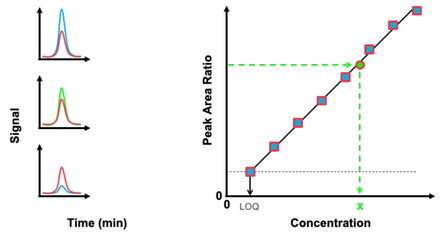


QUANTITATIVE DATA ANALYSIS

[A] Conventional External Curve

U.S. Food and Drug Administration Bioanalytical Method Validation. Guidance for Industry (2018)

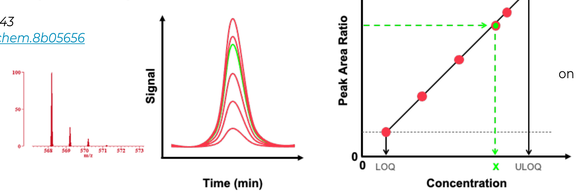
- External calibration curve
- Unknown sample



[B] Multiple Isotopologue Reaction Monitoring In-Sample Calibration Curve (MIRM-ISCC)

Anal. Chem. 2019, 91, 3, 2536-2543
https://doi.org/10.1021/acs.analchem.8b05656

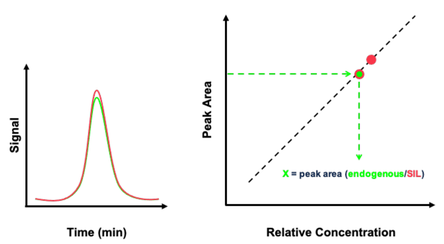
- Internal curve based the natural abundance distribution of the SIL peptide isotopologues
- Unknown sample



[C] Absolute Quantitation (AQUA)

PNAS June 10, 2003 100 (12) 6940-6945;
https://doi.org/10.1073/pnas.0832254100

- Single calibration point
- Unknown sample



RESULTS

PRECISION AND ACCURACY

Operator 1

	Calibration Curve				MRM (1/X Weighted)				AQUA Approach			
	QCLOQ	QC-0*	QC-2	QC-3	QCLOQ	QC-0*	QC-2	QC-3[&]	QCLOQ	QC-0*	QC-2	QC-3
Nominal	1.00	3.43	48.4	753.4	1.0	3.67	48.7	753.7	1.00	4.08	49.1	754.1
Experimental	1.04	3.35	36.3	828.8	1.20	3.50	41.9	993.0	1.16	3.98	44.8	1027.6
	1.22	3.51	44.8	832.1	1.18	3.83	50.9	916.5	1.36	4.18	55.5	1031.7
	1.16	N/AP	N/AP	N/AP	1.07	N/AP	N/AP	N/AP	1.31	N/AP	N/AP	N/AP
Mean	1.14	3.43	40.6	830.4	1.15	3.67	46.4	954.8	1.28	4.08	50.1	1029.6
% Accuracy	114.2%	N/AP	83.7%	110.2%	115.0%	N/AP	95.4%	126.7%	127.6%	N/AP	102.2%	136.5%
% CV	7.9%	3.2%	14.9%	0.3%	6.1%	6.4%	13.7%	5.7%	8.2%	3.5%	15.0%	0.3%
%Bias v s. Calibration Curv e	N/AP				0.7%	6.8%	14.4%	15.0%	11.8%	18.9%	23.6%	24.0%

Operator 2

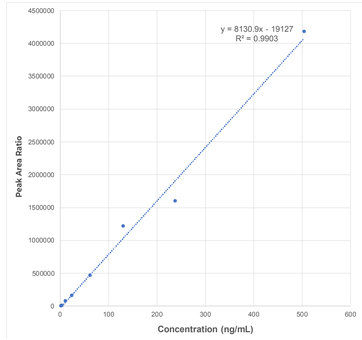
	Calibration Curve				MRM (1/X Weighted)				AQUA Approach			
	QCLOQ	QC-0*	QC-2	QC-3	QCLOQ	QC-0*	QC-2	QC-3[&]	QCLOQ	QC-0	QC-2	QC-3
Nominal	1.00	3.43	48.4	753.4	1.0	3.61	48.6	753.6	1.00	3.93	48.9	753.9
Experimental	0.99	3.64	41.6	821.7	0.80	3.48	41.3	810.8	0.91	3.78	44.7	885.6
	1.07	4.20	60.6	982.4	1.08	3.87	58.1	931.5	1.01	4.38	65.2	1058.9
	1.22	N/AP	N/AP	N/AP	1.35	N/AP	N/AP	N/AP	1.16	N/AP	N/AP	N/AP
Mean	1.09	3.92	51.1	902.0	1.08	3.68	49.7	871.2	1.02	4.08	55.0	972.3
% Accuracy	109.4%	N/AP	105.5%	119.7%	107.7%	N/AP	102.2%	115.6%	102.4%	103.8%	112.3%	129.0%
% CV	10.9%	10.0%	26.3%	12.6%	25.5%	7.5%	23.9%	9.8%	12.4%	10.5%	26.3%	12.6%
% Bias v s. Calibration Curv e	N/AP				-1.6%	-6.3%	-2.8%	-3.4%	-6.3%	4.0%	7.5%	7.8%

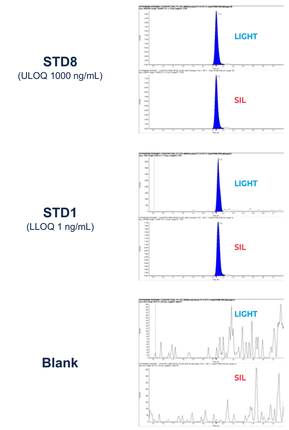
- Similar precision and accuracy between calibration curve and MIRM approach
- AQUA approach provided worse accuracy at QC3

CALIBRATION CURVES

Conventional External Curve

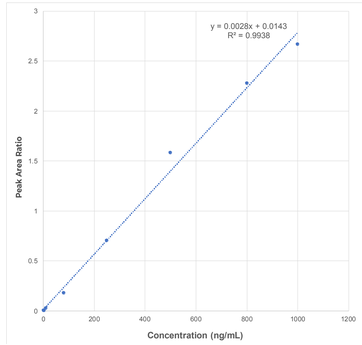
Evaluated range: 1 to 1000 ng/mL

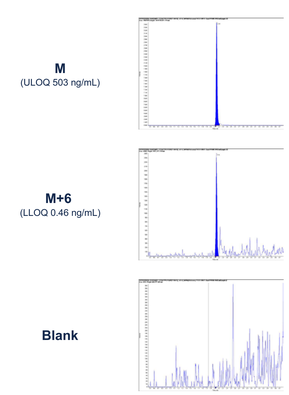




In-Sample Calibration Curve

Analytical range: 1 to 500* ng/mL





- **ULOQ =** SIL peptide standard spiking level, based on the monoisotopic peak (M). This concentration was set at 503 ng/mL to limit the contribution of the unlabeled standard to the light signal.
- **LLOQ =** Lowest abundant isotope (M+6) that could be quantified precisely and accurately.

CONCLUSION

- **We developed high-throughput mass spectrometry MRM platforms for quantitation of sBCMA in human plasma with ~3 day turnaround time**
- **We evaluated different approaches to quantitative data analysis and obtained comparable results for external calibration curve, ISCC-MIRM and AQUA**
- **These assays may be bioanalytically validated and deployed for clinical studies using the quantitation approach appropriate to the program stage and intended use of the data**
  - AQUA is appropriate for exploratory studies and allows for a high degree of multiplexing with other targets.
  - MIRM-ISCC shows promise for low-throughput clinical sample analysis under CAP/CLIA, where use of external curves and QC samples may be impractical.
  - A 'PK-like' approach should be used to fully assess assay robustness, if the biomarker is to be used as a PD marker.

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