Quantification of Albumin and Creatinine in Urine by MALDI-TOF Mass Spectrometry

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Microalbuminuria

•microalbuminuria = *Albumin in urine*

-Low levels of albumin in urine can be normal

-Temporarily high levels of albumin in urine aren't unusual either, particularly after exercise or during an illness

- Often *monitored in cases of diabetes* and newly developing or increasing amounts of albumin in urine may be an earliest sign of *diabetic kidney damage*.

Clinical Definitions:

Microalbuminuria	= 30 - 300 mg albumin / g creatinine
Macroalbuminuria	> 300 mg/g

Creatinine and [Albumin]/[Creatinine] Ratio

- The *concentration (or dilution) of urine varies* throughout the day with more or less liquid being released in addition to the body's waste products. Thus, the *concentration of albumin in the urine may also vary*.
- **Creatinine** is a waste product from the normal breakdown of muscle tissue, it is filtered through the kidneys, and excreted in urine.
- **Creatinine** is *normally released into the urine at a constant rate* and its level in the urine is an indication of the urine concentration.
- To compensate for variations in urine albumin concentration it is helpful to compare its concentration against that of creatinine.
- Typically reported as a ratio [albumin]/[creatinine]

American Association of Clinical Chemisty <https://www.aacc.org>

How are Albumin and Creatinine currently Quantified by Clinical Chemists

- [Albumin] is measured by a *turbidimetric* method:
  - albumin combines with specific antibody to form insoluble antigen-antibody complexes

Albumin + Anti-albumin Antibody → Antigen-antibody Complex

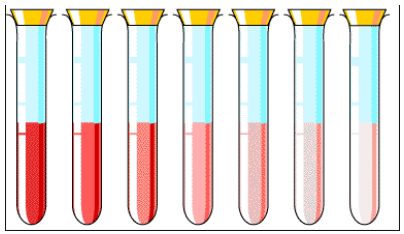
- Range: 0.2 -30 mg/dL
- Precision : 1.0 mg/dL CV = 8.7% within run and 12.2% total
  39.3 mg/dL CV = 1.6 % within run and 1.8 % total

- [Creatinine]is measured by *colorimetric* method of Jaffe(1880

Creatinine + Picric Acid ⟶ Creatinine-Picric Complex (red)

- Color development monitored at λ 520nm
- Range in urine 10 – 400 mg/dL
- Precision CV = 3%

Quantification of Albumin in Urine Using MALDI-TOF MS



Create Calibration Curve with spiked albumin and internal standard

-- Albumin standard weighed and diluted with pooled urine to 100 µM

-- 10x dilution of pooled Std with individual control sample to create 10 µM

-- Dilute 10µM by 2x six times using same urine sample
span clinically range 10.00 to 0.31 µM

--All standard further diluted 1:10 (sample:matrix) containing internal std.
0.075 µM (cytochrome C or lysozyme) in 10 mg/mL alpha-cy 75% CH_3CN, 0.1% TFA

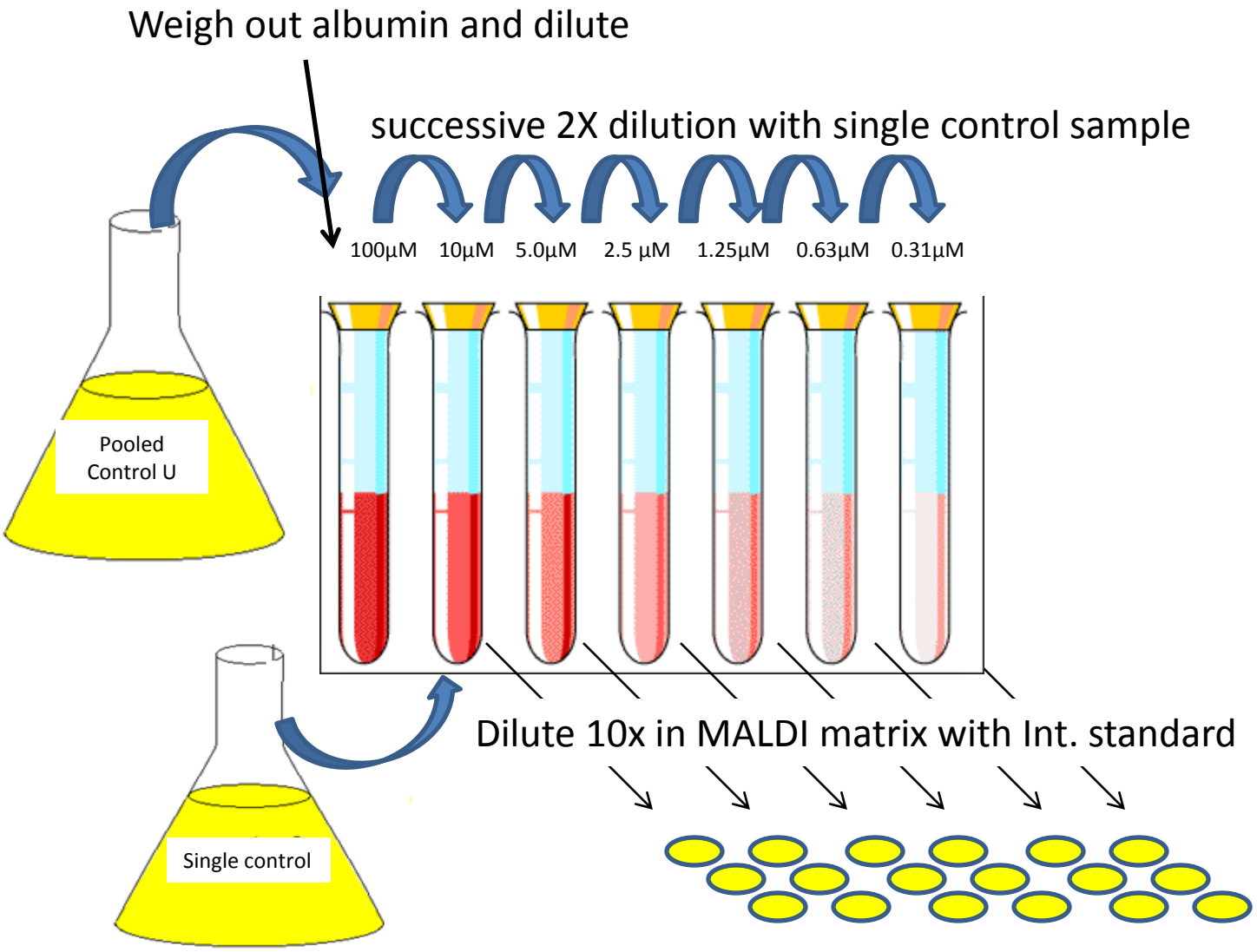
-- All concentrations spotted in 6x replication

--All spectra normalized to internal standard singly charged peak

-- Integration albumin response used to create calibration curve for quantitation of unknowns.

Patient sample diluted 10x with matrix and internal standard
-run in 3 x replication
-all spectra normalized to internal standard signal
-signal quantitated using calibration curve constructed from control

Create dilution series of Albumin in Control Urine



MALDI-TOF MS

- SimulTOF ONE MALDI-TOF mass spectrometer

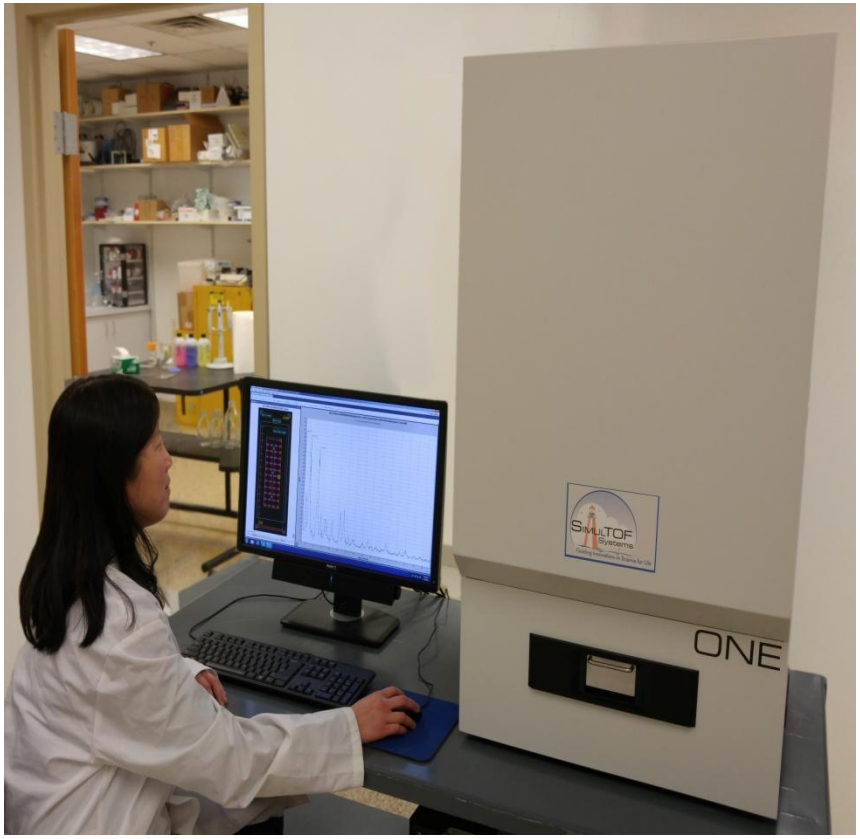
Capabilities:

- Max accelerating voltage 20 kV

- Max laser pulse frequency 5000 Hz

- Max scan speed 10 mm/s

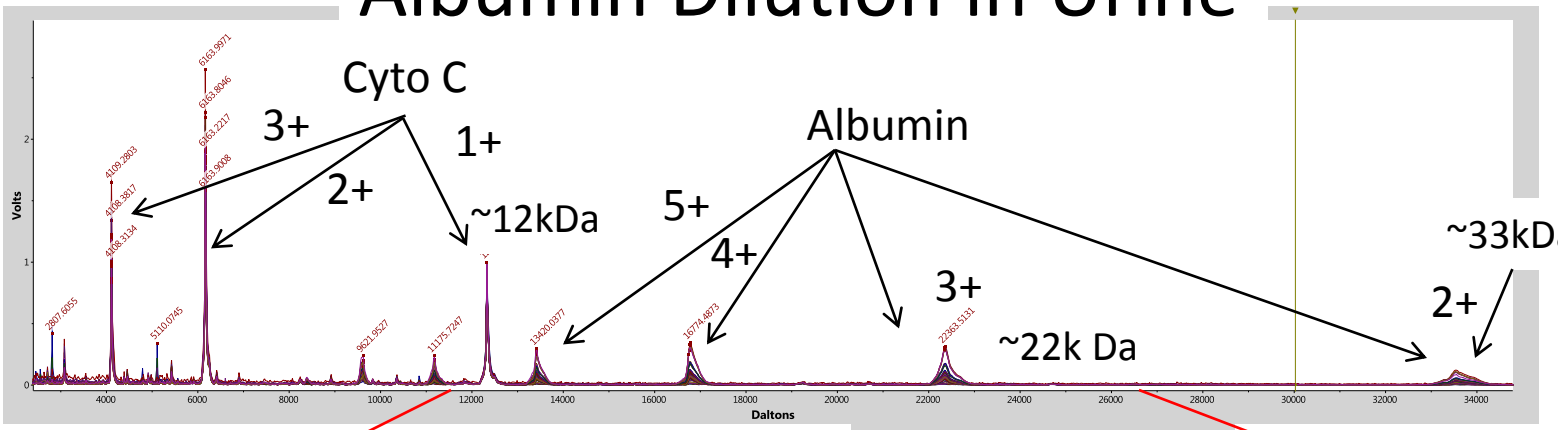
- Mass range 100 – 1,000,000 Da

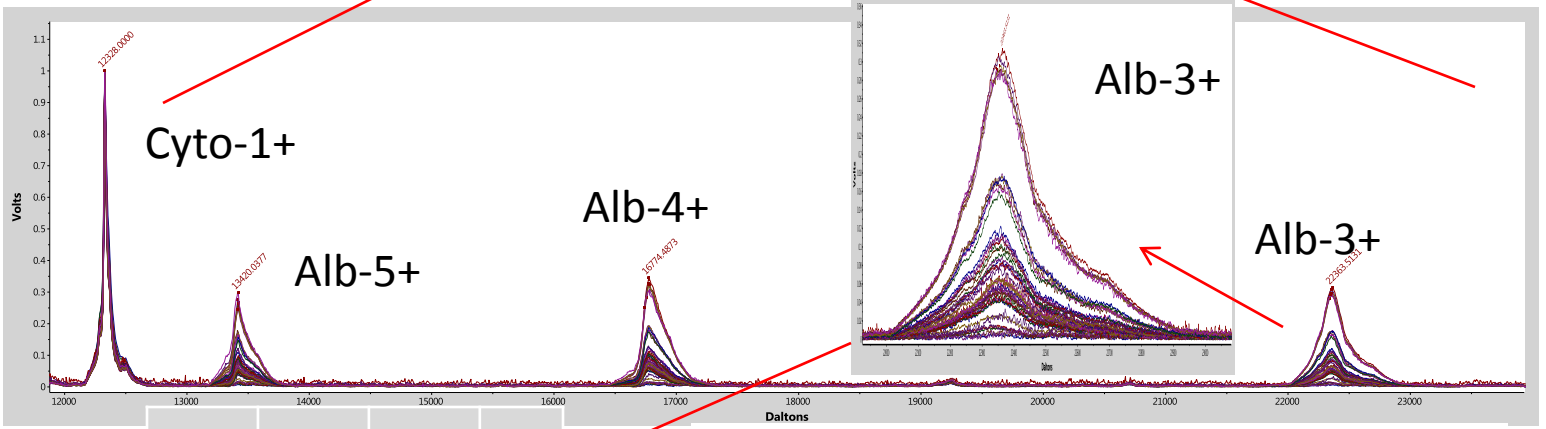


<u>Acquisition parameters</u>

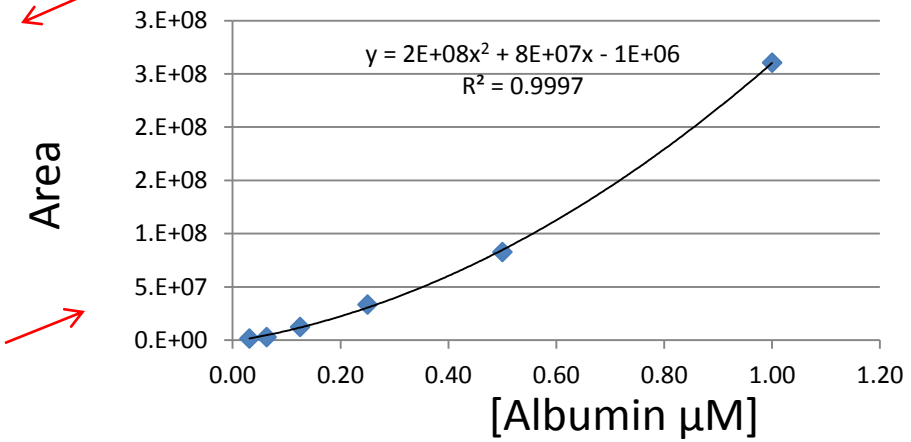
- Linear mode: positive-ion polarization
- Focus mass 20,000 and 120 Da
- Laser pulse frequency 1000 Hz
- Laser pulse energy 3 and 1.8 µJ
- Scan rate 1 mm/s
- Spot size 2.6 µm
-100 µm raster to cover each sample position

Albumin Dilution in Urine





[Albumin]	Area	Std Dev	CV %
0.03	1.55E+06	1.02E+05	6.58
0.06	2.90E+06	1.58E+05	5.43
0.13	1.24E+07	1.01E+06	8.12
0.25	7.63E+07	2.27E+06	6.78
0.50	8.27E+07	5.10E+06	6.16
1.00	2.60E+08	1.53E+07	5.86



Quantification of Creatinine Using Isotopically labeled Creatinine (d3)

Established quantitative response across clinically relevant range using control

-Deuterated creatinine (d3) spiked into urine at 4 different concentrations to span clinically relevant range

-Samples analyzed and normalized to native creatinine peak

-Calibration curve constructed based and spiked [creatinine] vs. peak area for d3 peak

-preformed at urine dilution of 100x

Performed relative quantitation in unknowns using a single point spike

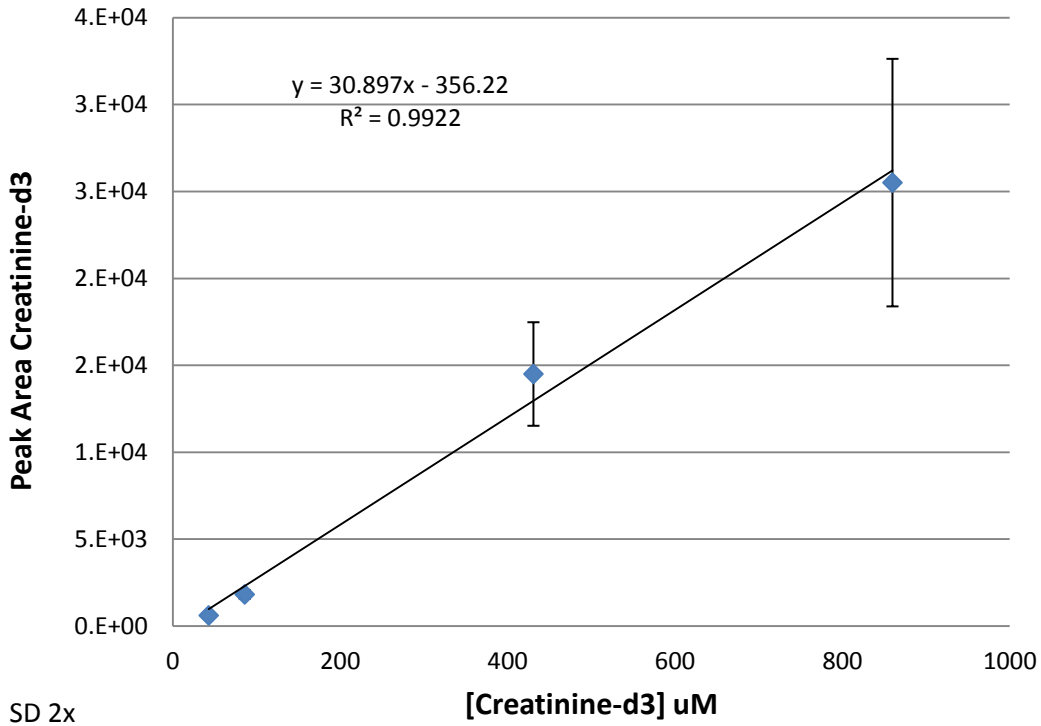
-With signal response calibrated across clinically relevant range

-[Creatinine] in unknowns was measured by relative comparison of native creatinine signal to a single (mid-range) spike of creatinine-d3 included in MALDI matrix

-preformed at urine sample dilution of 10x

Normal range urine (male)
3.5-26.5 mM

urine diluted 100x
35 – 265 µM

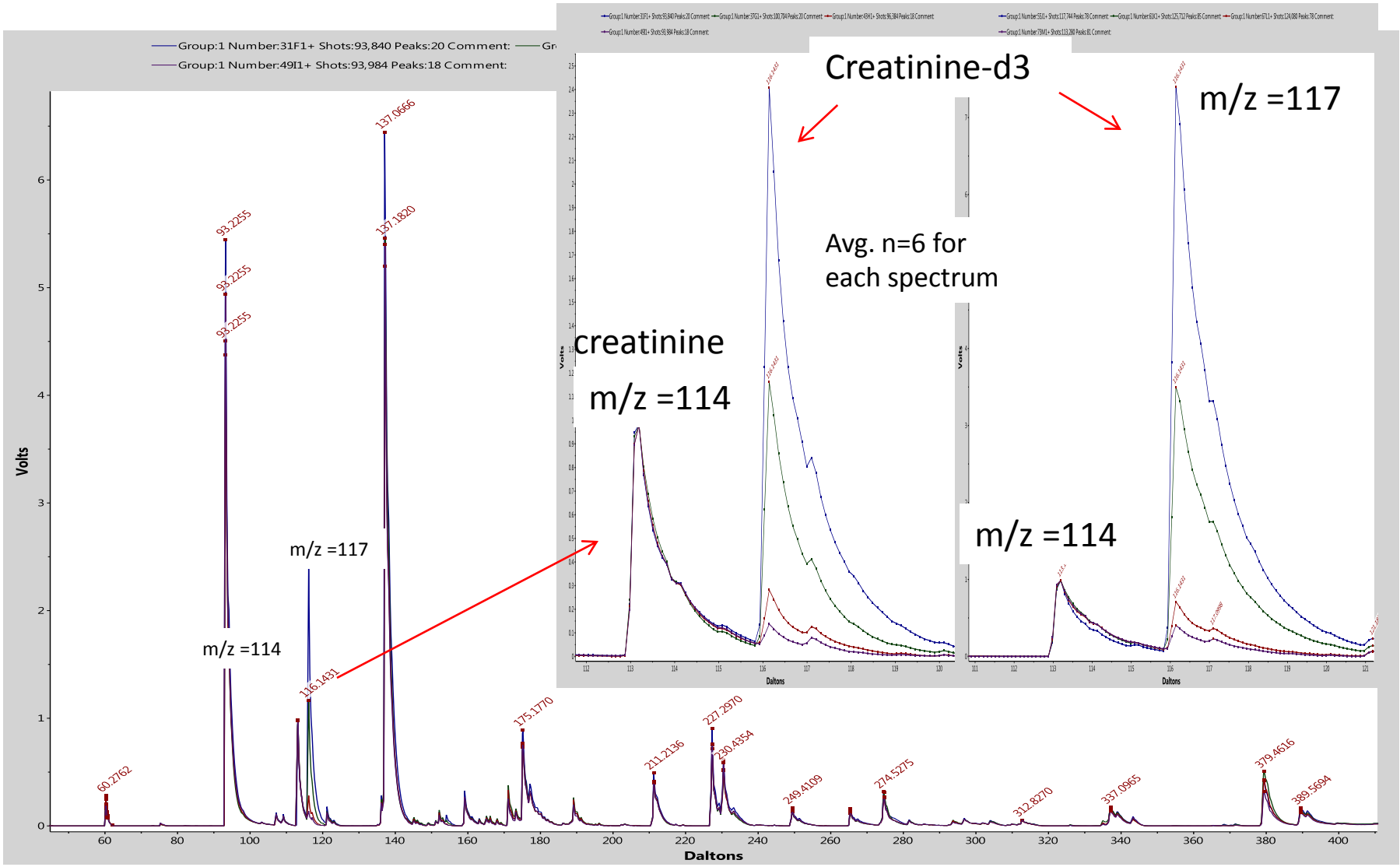


Error bars ± 2x Std Dev

[Creatinine-d3]	Area (n=6)	SD	CV	SD 2x
43.00	615.10	70.07	11.39	140.13
86.00	1823.91	123.38	6.76	246.76
431.00	14501.29	1488.90	10.27	2977.80
860.00	25508.39	3559.71	13.96	7119.42

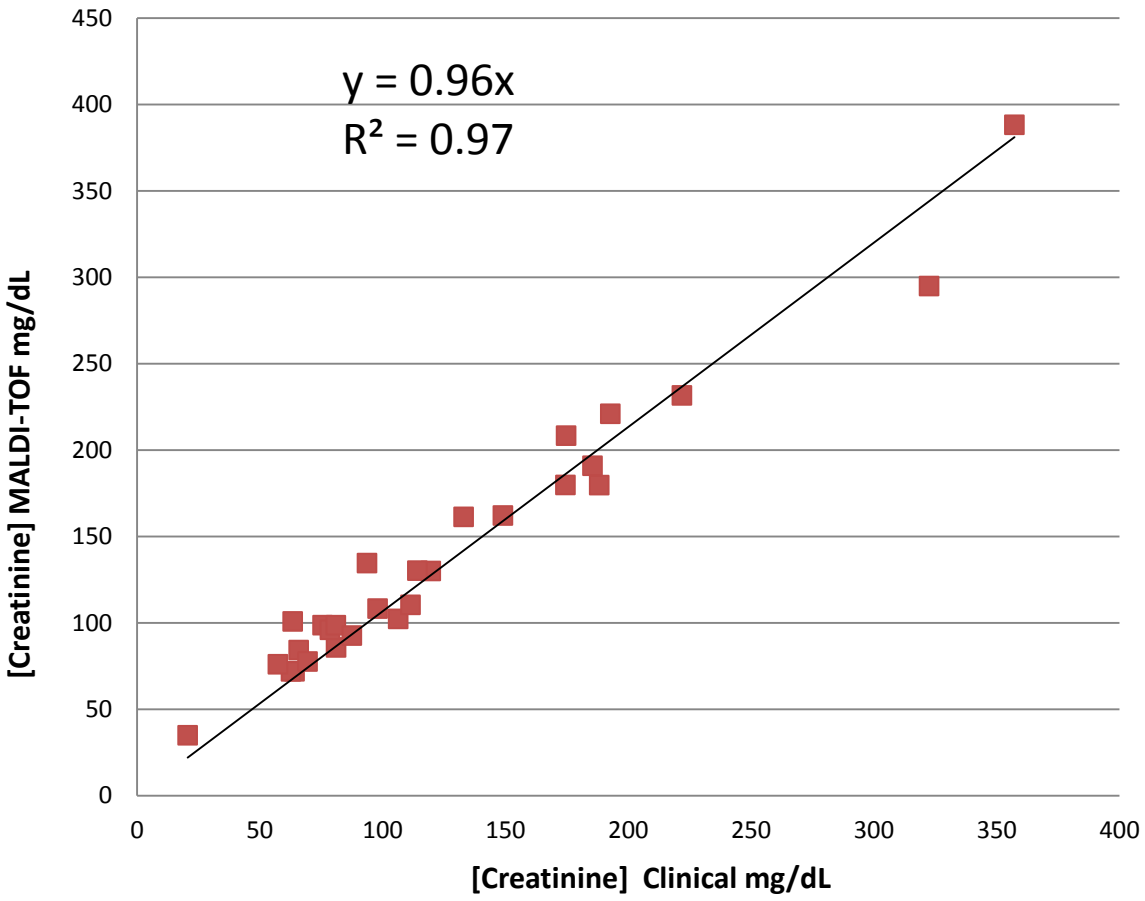
	Area 113.14 (n=6)	SD	CV	[Calculated]
T1	10246.81	381.05	3.71	343.14
T2	11137.06	980.24	8.80	371.95
T3	10816.40	391.24	3.61	361.57
T4	10941.94	806.46	7.37	365.64
		Avg		360.58
		SD		12.38
		CV %		3.43

Creatinine overlay of avg. spectra for calibration curve



Comparison of [Creatinine] Clinical vs. MALDI-TOF relative quantitation against 9 mg/dL Creatinine d3 spike

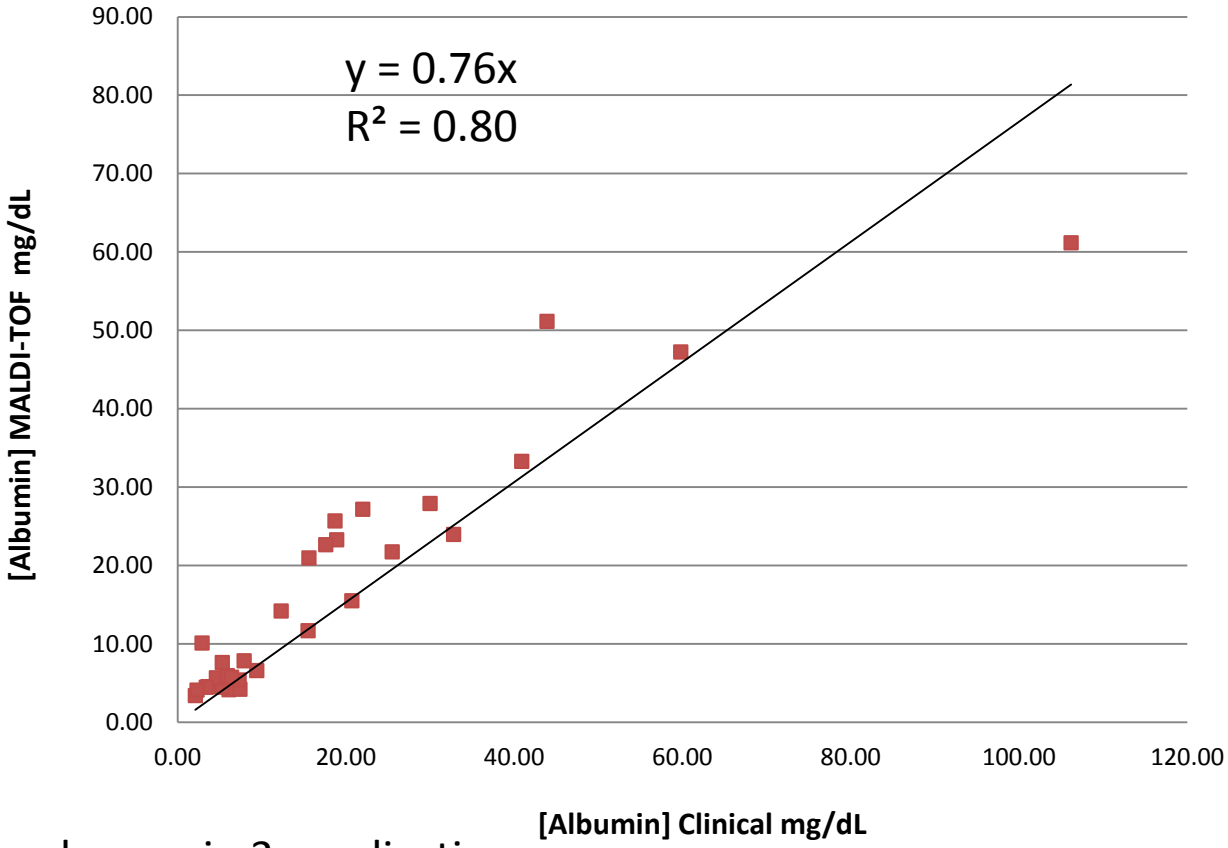
Sample	Clinical mg/dL	MALDI mg/dL	CV %
1			
2			
3	63.4	100.8	1.1
4	192.7	221.0	3.3
5	93.6	134.6	7.8
6	98.0	108.3	3.8
7	119.6	130.0	3.6
8	65.8	84.3	1.4
9	81.0	85.8	0.7
10	75.5	98.7	2.1
11	64.1	72.0	1.4
12	62.7	71.9	2.7
13	221.9	231.7	1.5
14	188.2	179.7	1.1
15	357.3	388.3	2.6
16	114.2	130.2	0.9
17	174.4	179.8	5.0
18	133.0	161.4	3.5
19	185.5	190.9	3.9
20	149.0	162.1	2.6
21	78.5	96.0	1.7
22	322.5	294.9	6.1
23	174.7	208.4	2.0
24	80.9	98.7	9.0
25	106.3	102.3	0.2
26	111.4	110.4	0.9
27	69.4	77.6	3.5
28	87.5	92.7	0.8
29	57.3	76.1	1.2
30	20.6	34.9	4.7
		Avg. =	2.81



Measurements done in 3x replication

Comparison of [Albumin] Clinical vs. MALDI-TOF

Sample	Clinical mg/dL	MALDI mg/dL	CV %
1	3.6	4.6	14.5
2	6.1	4.2	17.5
3	43.9	51.2	31.7
4	5.3	4.5	3.3
5	18.9	23.3	28.4
6	2.1	3.4	17.8
7	32.8	24.0	24.8
8	4.6	5.7	37.5
9	9.4	6.6	34.2
10	22.0	27.2	17.5
11	25.5	21.8	17.8
12	7.4	4.2	12.1
13	20.7	15.5	36.4
14	15.5	11.7	18.8
15	5.9	6.0	33.1
16	7.3	5.4	10.8
17	3.4	4.5	10.1
18	17.6	22.7	11.4
19	7.9	7.9	27.2
20	12.3	14.2	22.0
21	18.7	25.7	3.2
22	6.4	5.8	4.3
23	106.2	61.2	30.2
24	2.9	10.1	37.3
25	59.8	47.3	9.2
26	2.3	4.1	5.4
27	40.9	33.3	20.9
28	5.3	7.6	13.6
29	30.0	27.9	24.7
30	15.6	21.0	2.7
		Avg. =	19.51

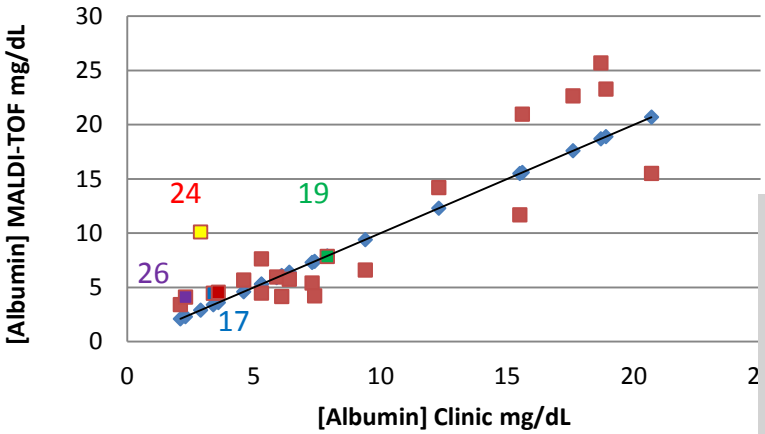


All samples run in 3x replication

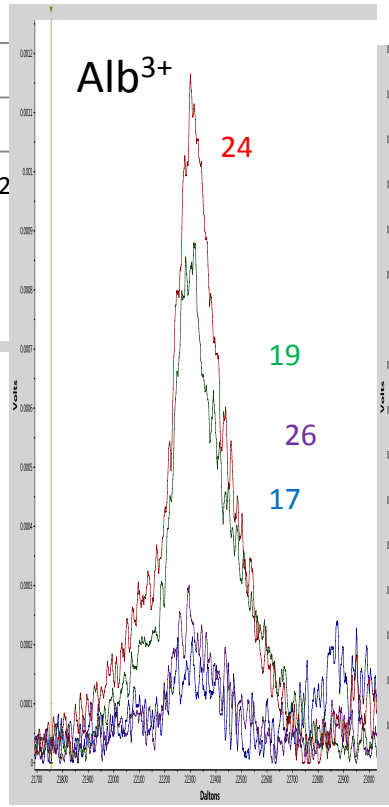
Wednesday 3:00 PM ; ***Proteomics***
"A Reference Measurement System for Urine Albumin"
Ashley Beasley Green, NIST

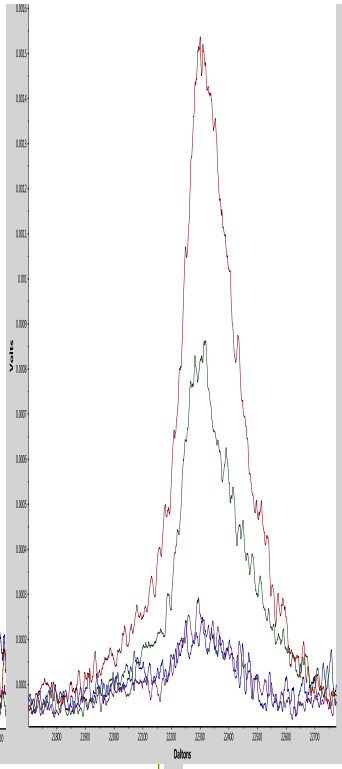
Closer look at low end measurements of [Albumin]

Sample #	[Albumin] Clinical	[Albumin] MALDI-TOF
6	2.1	3.4
26	2.3	4.1
24	2.9	10.1
17	3.4	4.5
1	3.6	4.6
8	4.6	5.7
4	5.3	4.5
28	5.3	7.6
15	5.9	6.0
2	6.1	4.2
22	6.4	5.8
16	7.3	5.4
12	7.4	4.2
19	7.9	7.9
9	9.4	6.6
20	12.3	14.2
14	15.5	11.7
30	15.6	21.0
18	17.6	22.7
21	18.7	25.7
5	18.9	23.3
13	20.7	15.5

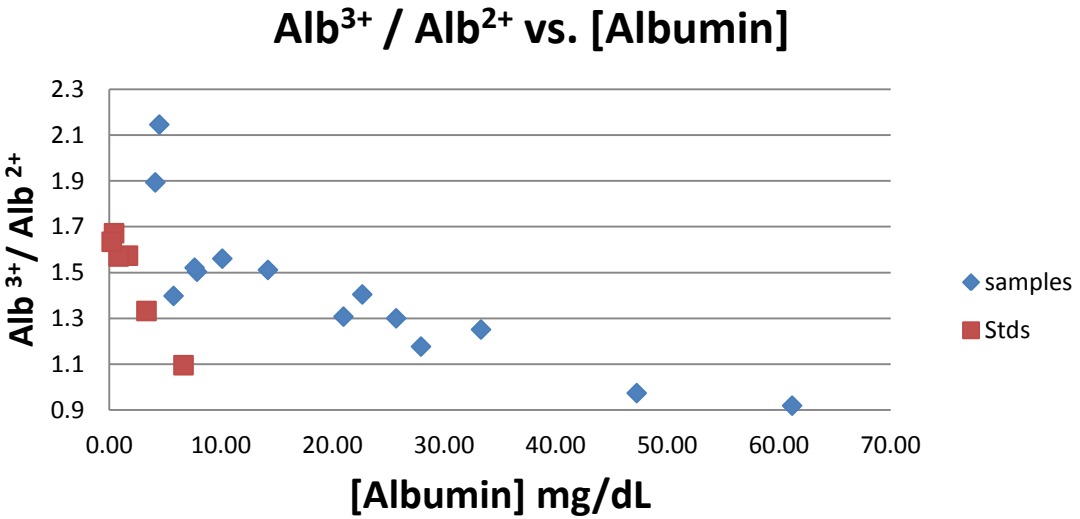


No normalization

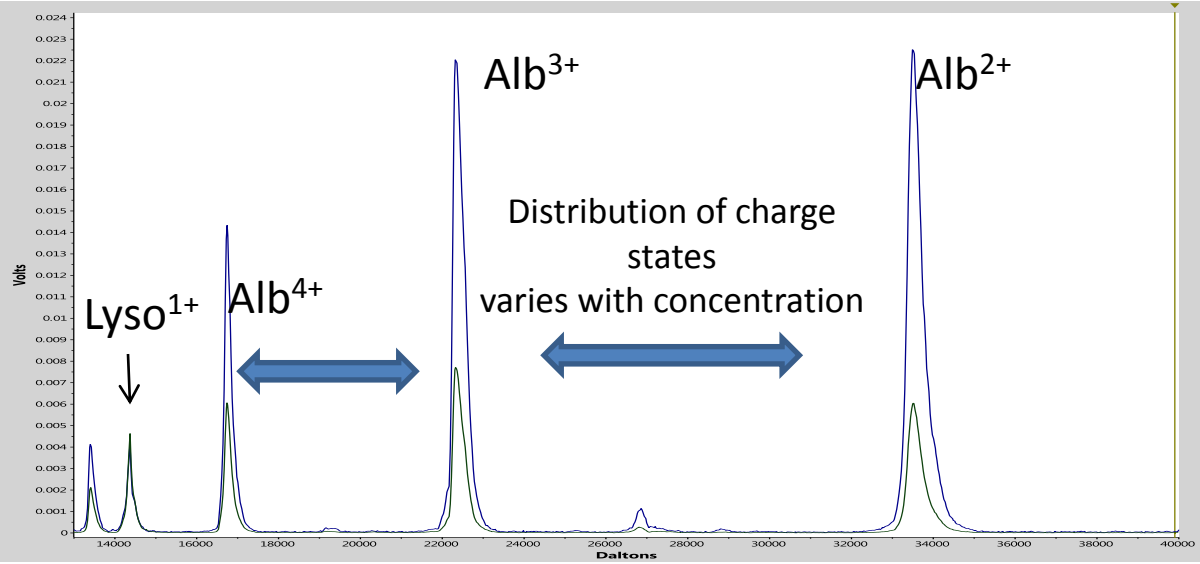




As [Albumin] increases the signal distribution between charged states changes

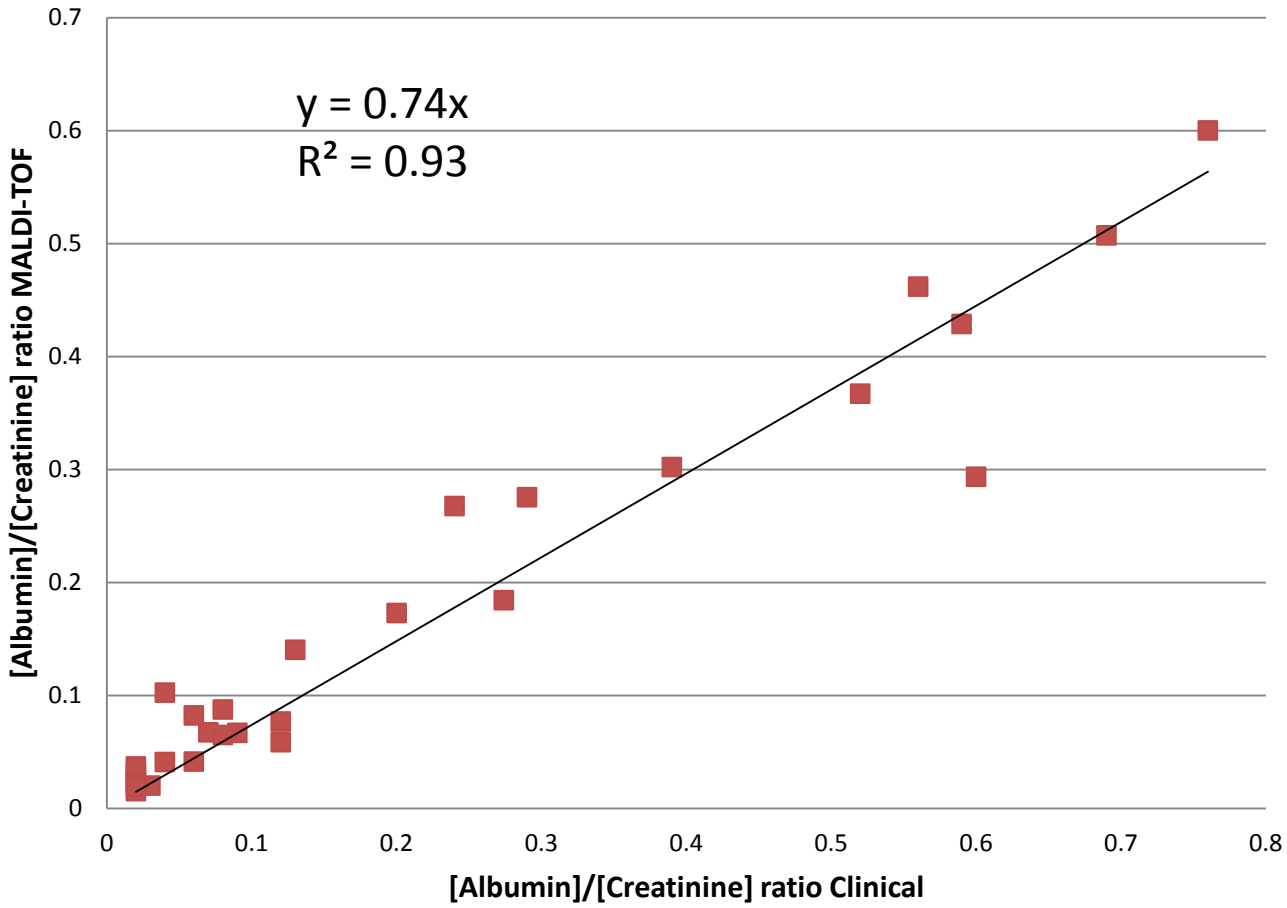


impacting [albumin] calculation ?



Comparison of Albumin/Creatinine ratio Clinic vs. MALDI-TOF (mg/mg)

Sample	Clinical Ratio	MALDI Ratio
1		
2		
3	0.69	0.51
4	0.03	0.02
5	0.20	0.17
6	0.02	0.03
7	0.27	0.18
8	0.07	0.07
9	0.12	0.08
10	0.29	0.28
11	0.39	0.30
12	0.12	0.06
13	0.09	0.07
14	0.08	0.07
15	0.02	0.02
16	0.06	0.04
17	0.02	0.02
18	0.13	0.14
19	0.04	0.04
20	0.08	0.09
21	0.24	0.27
22	0.02	0.02
23	0.60	0.29
24	0.04	0.10
25	0.56	0.46
26	0.02	0.04
27	0.59	0.43
28	0.06	0.08
29	0.52	0.37
30	0.76	0.60



Conclusions

- MALDI-TOF MS capable of quantify albumin and creatinine across clinically relevant ranges
  - -Work to do on measurement variance
    - -internal standard ~ the target analyte
    - -investigate sample formulation
- Single 10x dilution of urine get both values from the same spot
- Direct measurement of analyte in question
- Minutes / analysis with multiple replicates
- Auxiliary information available