A Soluble Receptor (sBCMA) Biomarker Parallelism Case Study

—Using Parallelism Experiments to Effectively Evaluate Matrix Effects and Selectivity in Early Stage LBA Method Development

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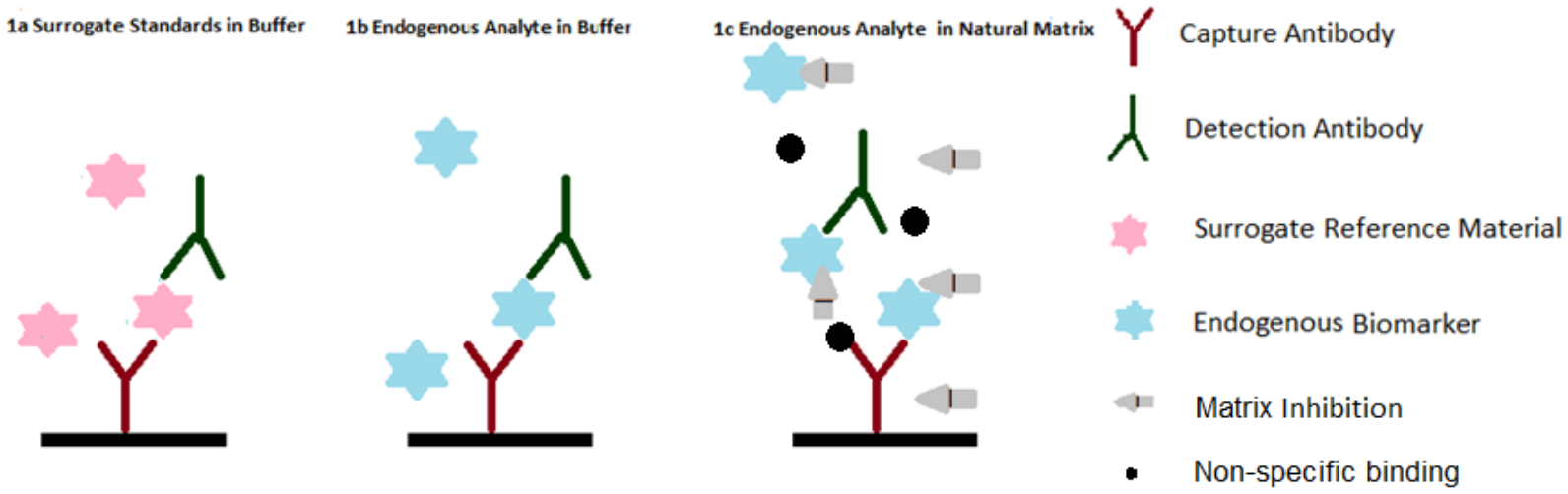
Outline

1. Challenges for Biomarker LBAs
2. Parallelism Experiment
3. sBCMA Case Study
4. Parallelism and Specificity
5. Acknowledgements

Challenges for Biomarker LBAs

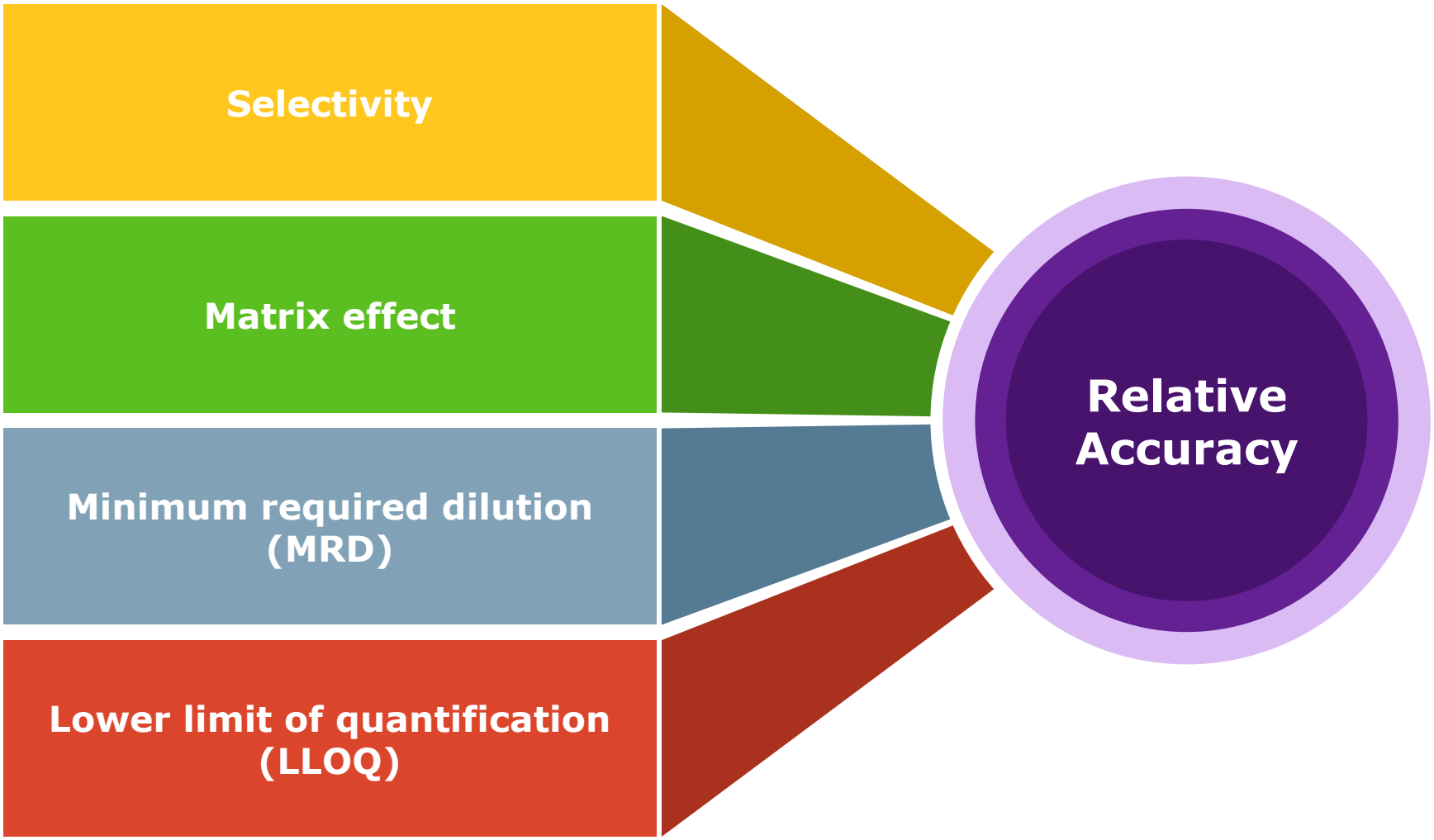


- Lack of natural matrix free of endogenous analyte
- Lack of well-characterized reference standards
- Potential mismatch of recombinant surrogate reference material with actual endogenous analyte form
- Potential matrix interference
- Potential specificity issues

Parallelism Experiment

- Evaluate parallelism between surrogate reference material fortified in surrogate matrix and natural protein serially diluted with surrogate matrix
  - Prepare calibrators in surrogate matrix calibrator diluent
  - Analyze sample neat and serially diluted with calibrator diluent
  - Plot dilution-adjusted concentrations vs. 1/dilution factor to evaluate method parallelism

What do parallelism data tell us?



sBCMA Case Study

- sBCMA, also known as Tumor Necrosis Factor Receptor Superfamily member 17 (TNFRSF17), is a cell surface receptor of the TNF receptor superfamily.
- sBCMA has been detected at ng/mL concentrations in the serum of healthy subjects and multiple myeloma (MM) patients
- sBCMA desired bioanalytical assay standard curve quantification range was 15.6-4000 pg/mL
- The endogenous sBCMA level is high enough to allow for measurement of multiple dilutions across the assay range

Before Dilution Buffer Optimization

- 1% BSAPBS was evaluated as surrogate matrix
- 10 individual lots of normal human sera (5 male and 5 female) were serially diluted 1:4 with 1%BSA PBS buffer.

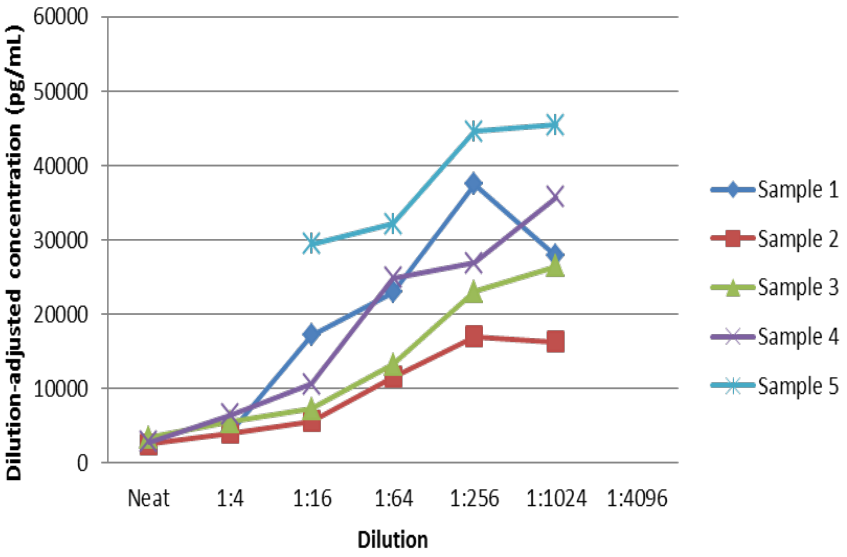
Sample dilution	Human Serum Lot Dilution-adjusted concentration, pg/mL (% bias from 1:1024 dilution results)									
	1	2	3	4	5	6	7	8	9	10
Neat	AQL	2518 (-85)	3417 (-87)	2804 (-92)	AQL	AQL	AQL	3736 (-85)	AQL	AQL
1:4	4113 (-85)	4015 (-75)	5508 (-79)	6512 (-82)	AQL	6155 (-85)	*16510 (-71)	7651 (-70)	AQL	12819 (-60)
1:16	17126 (-38)	5561 (-66)	7298 (-72)	10578 (-70)	29427 (-35)	11570 (-72)	20858 (-64)	9846 (-62)	16081 (-48)	11794 (-63)
1:64	22906 (-18)	11562 (-29)	13244 (-50)	24864 (-30)	32129 (-29)	22102 (-46)	31879 (-44)	16100 (-37)	22724 (-27)	19043 (-41)
1:256	37504 (35)	17011 (5)	23051 (-13)	26896 (-25)	44553 (-2)	32465 (-21)	45319 (-21)	25563 (0)	26619 (-14)	30229 (-6)
1:1024	27830	16252	26442	35723	45482	41093	57409	25655	31091	32110
1:4096	33067	N/A	BQL	BQL	41705	40894	55882	N/A	32227	36811

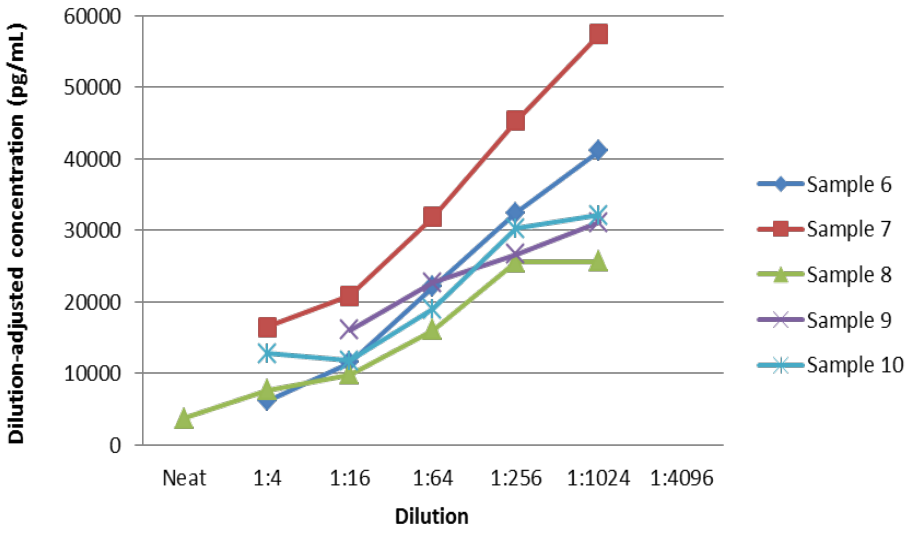
Notes:

*** Samples have values above curve ULOQ, extrapolated values provided for information only**

AQL Above Quantifiable Limit
BQL Below Quantifiable Limit

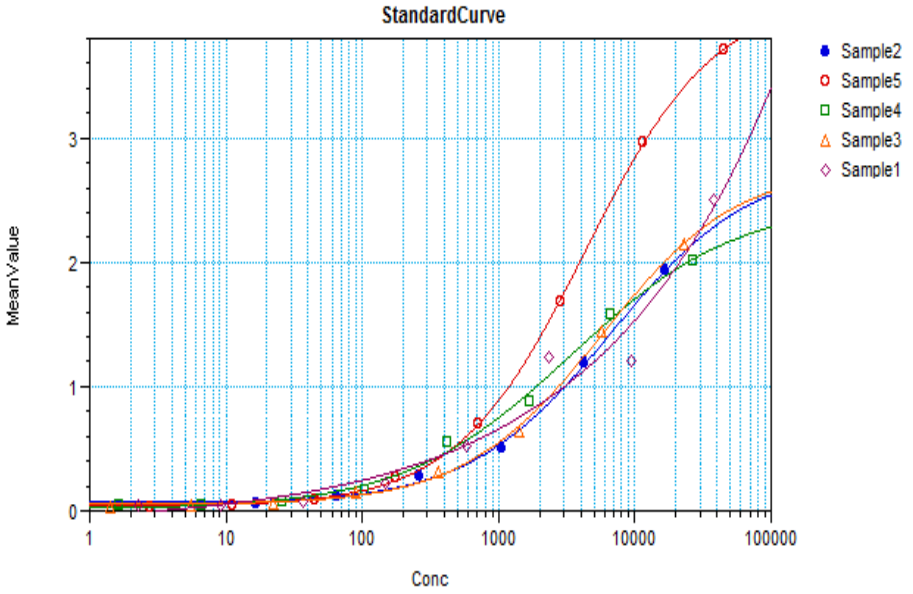
Before Dilution Buffer Optimization

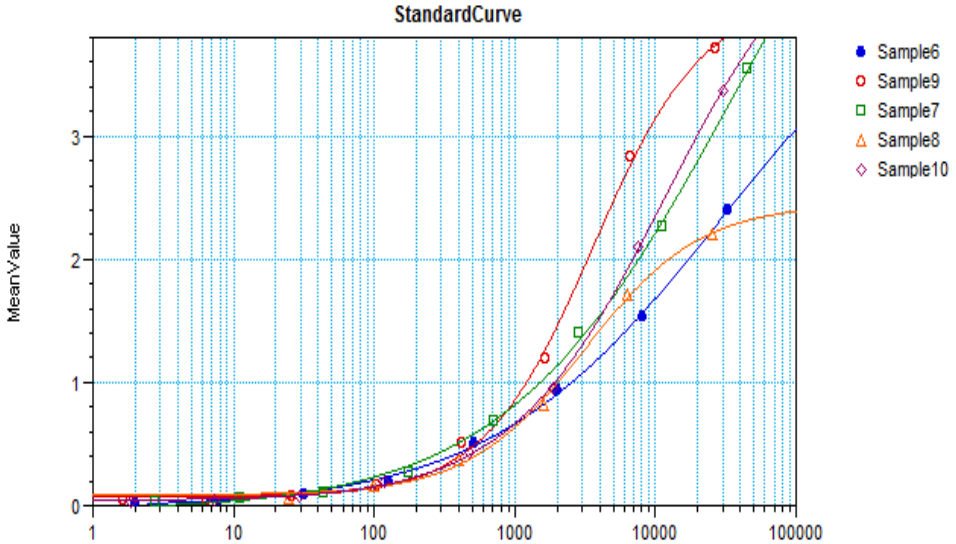




- No parallelism was observed between calibrators and samples
- The data suggested strong matrix inhibition

Before Dilution Buffer Optimization





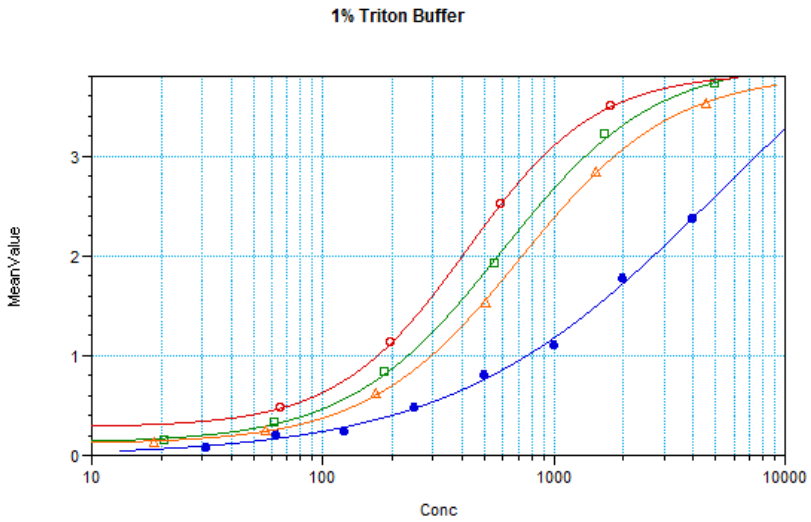
- Sample data was evaluated using a 4 parameter logistic (4PL) regression with SoftMax® Pro software (Molecular Devices)
- No parallelism was observed among different samples
- Data suggested selectivity issue - varying levels of matrix interference exist in different samples.

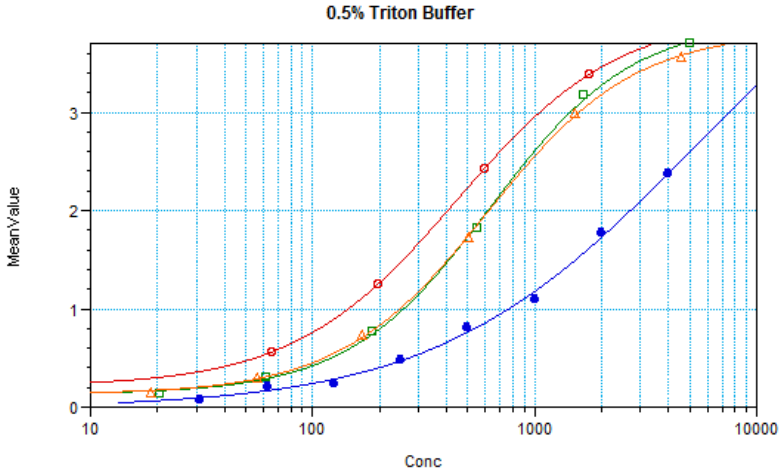
Dilution Buffer Optimization

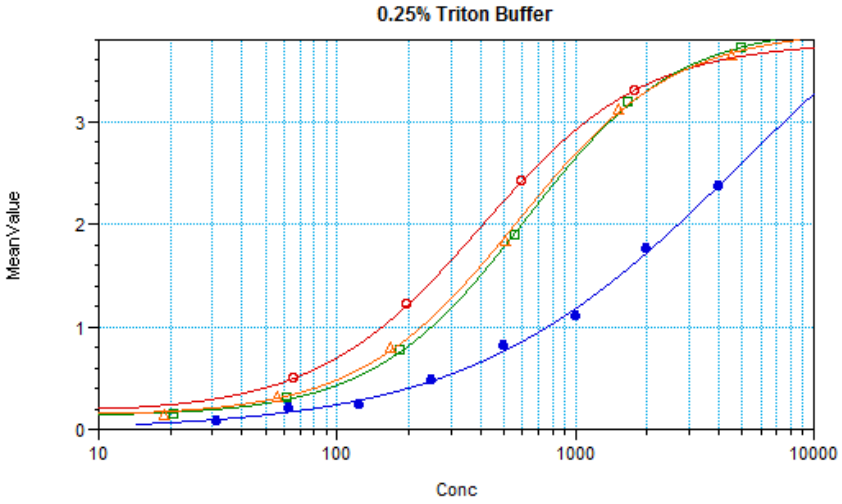
- Modified 1%BSA PBS buffers were evaluated containing Triton X-100 at 0.25%, 0.5%, and 1% (v/v), respectively
- Three human serum lots (2, 5 and 6) were 1:3 serially diluted with Triton buffers
- Standard curve was prepared in 1%BSA PBS

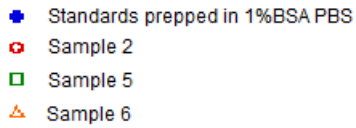
Trion X-100 Conc.	Optical Density (OD)								
Sample dilution	Lot 2			Lot 5			Lot 6		
	0.25%	0.50%	1.00%	0.25%	0.50%	1.00%	0.25%	0.50%	1.00%
Neat	2.2101	2.0109	2.0653	3.7791	3.7715	3.8177	2.5175	2.291	2.6388
1:3	3.8902	3.8836	3.6332	3.889	3.8674	3.8888	3.7399	3.7683	3.8271
1:9	3.5094	3.3887	3.3165	3.7259	3.7002	3.7509	3.5318	3.5665	3.6647
1:27	2.5153	2.4218	2.4114	3.2279	3.1668	3.1912	2.8438	2.982	3.1276
1:81	1.0989	1.2319	1.1904	1.9056	1.8132	1.8712	1.5118	1.7034	1.8217
1:243	0.4367	0.5383	0.4607	0.807	0.7412	0.7444	0.5857	0.7028	0.7721
1:729	N/A	N/A	N/A	0.2946	0.2895	0.2681	0.2184	0.2693	0.2796
1:2187	N/A	N/A	N/A	0.1231	0.1022	0.117	0.0902	0.1079	0.1125

Dilution Buffer Optimization





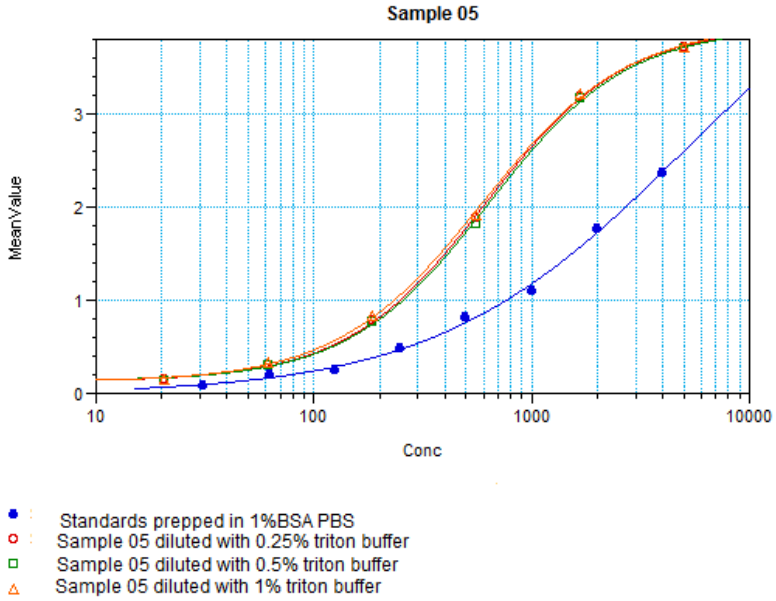




4 parameter logistic (4PL) regression was applied to evaluate the data

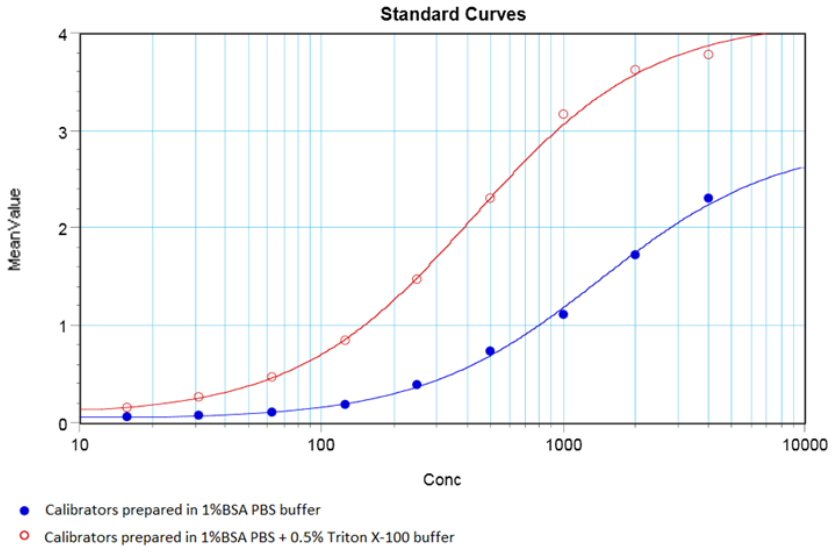
OD signals for samples that diluted 1:9 or greater were plotted against assigned theoretical concentrations

Dilution Buffer Optimization



- Parallelism was observed among samples at all Triton levels, but mismatched calibrators without

- Calibrator performance much improved in 1%BSA PBS buffer with 0.5% Triton X-100



After Dilution Buffer Optimization

- 0.5% Triton1% BSA PBS was selected as surrogate matrix

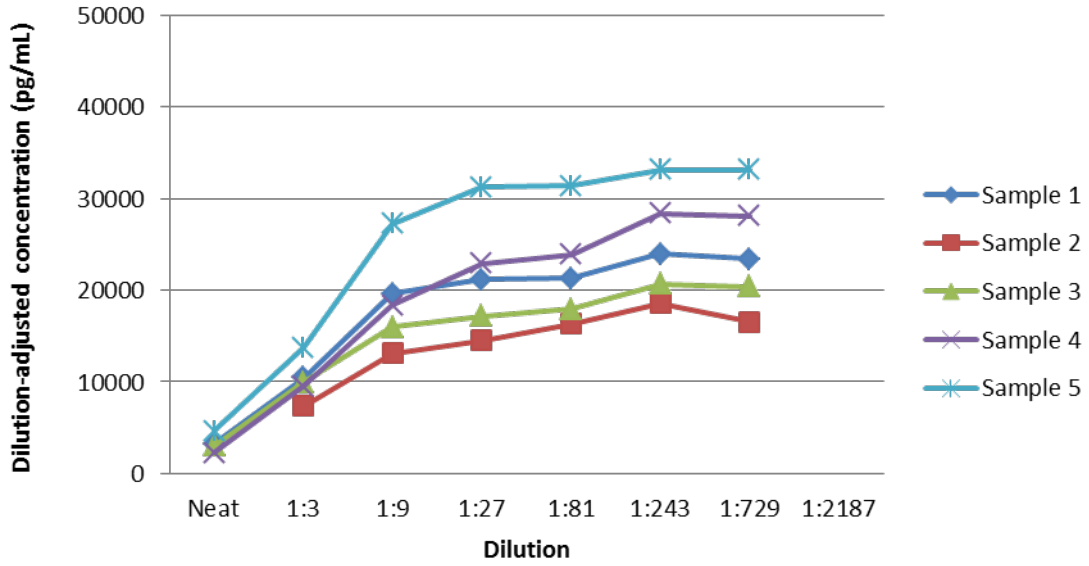
Sample Dilution	Human Serum Lot Dilution-adjusted concentration, pg/mL (% bias from 1:729 dilution results)									
	1	2	3	4	5	6	7	8	9	10
Neat	3249 (-85)	N/A	3038 (-85)	2268 (-92)	*4614 (-86)	2564 (-89)	*4046 (-89)	2823 (-90)	N/A	3793 (-84)
1:3	10459 (-55)	7379 (-55)	9974 (-51)	9534 (-66)	*13713 (-59)	9841 (-59)	*13100 (-66)	9920 (-63)	9472 (-62)	10747 (-56)
1:9	19608 (-16)	13144 (-20)	15996 (-22)	18381 (-35)	27253 (-18)	19426 (-20)	32289 (-15)	17409 (-35)	19044 (-23)	19810 (-18)
1:27	21151 (-9)	14479 (-12)	17137 (-16)	22889 (-19)	31201 (-6)	21586 (-11)	39372 (4)	21764 (-19)	19368 (-22)	21629 (-10)
1:81	21284 (-9)	16341 (-1)	17910 (-12)	23856 (-15)	31364 (-5)	24797 (3)	37889 (0)	24199 (-10)	22032 (-11)	22455 (-7)
1:243	23962 (3)	18584 (12)	20687 (1)	28391 (1)	33095 (0)	25304 (5)	39462 (4)	27414 (2)	25944 (5)	24255 (0)
1:729	23370	16523	20392	28118	33097	24181	38037	26912	24777	24158
1:2187	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL

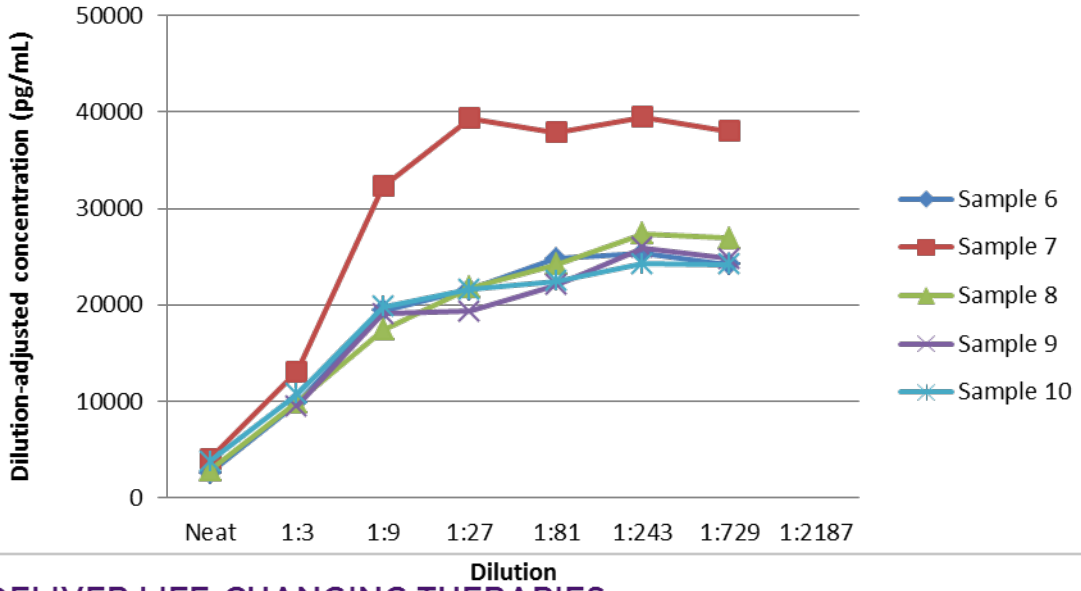
Notes:

***** **Samples have values above curve ULOQ, extrapolated values provided for information only**

BQL **Below Quantifiable Limit**

After Dilution Buffer Optimization





Parallelism and LLOQ

- Shall we use spike/recovery or parallelism experiment to evaluate assay LLOQ?

- What is the true LLOQ of the assay if we spike on top of the endogenous level?

  Endogenous conc. + spiked conc.?

  Or spiked conc.?

Parallelism, MRD, and LLOQ

- Use parallelism to evaluate relative accuracy between different dilutions
- Set MRD where most of the samples measure in assay range and multiple dilutions beyond the MRD yield accurate results
- Set assay LLOQ above the lowest concentration that has acceptable relative accuracy

Sample dilution	Human Serum Lot Concentration after dilution, pg/mL (% bias from MRD 1:27 results adjusted by dilution factor)									
	1	2	3	4	5	6	7	8	9	10
1:27	783.384	536.263	634.695	847.745	1155.591	799.469	1458.221	806.082	717.324	801.086
1:81	262.771 (0.6)	201.742 (12.9)	221.117 (4.5)	294.52 (4.2)	387.206 (0.5)	306.138 (14.9)	467.762 (-3.8)	298.752 (11.2)	271.995 (13.8)	277.228 (3.8)
1:243	98.611 (13.3)	76.478 (28.4)	85.133 (20.7)	116.834 (24.0)	136.194 (6.1)	104.131 (17.2)	162.397 (0.2)	112.815 (26.0)	106.767 (34.0)	99.816 (12.1)
1:729	32.057 (10.5)	22.665 (14.1)	27.973 (19.0)	38.57 (22.8)	45.4 (6.1)	33.17 (12.0)	52.177 (-3.4)	36.917 (23.7)	33.987 (27.9)	33.138 (11.7)
1:2187	7.034 (-27.3)	out of range	5.425 (-30.8)	9.415 (-10.0)	13.371 (-6.3)	7.637 (-22.6)	14.076 (-21.8)	8.345 (-16.1)	out of range	7.261 (-26.6)

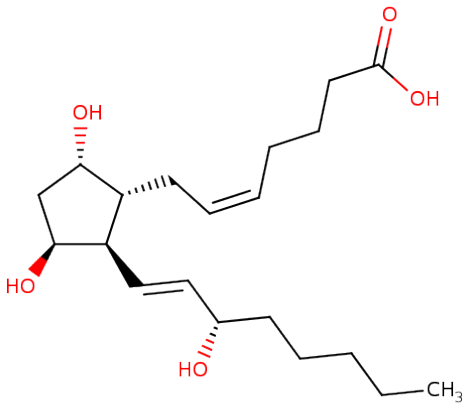
Parallelism and Specificity

- A recent case study - 9a,11b-PGF2a in human urine
  - LC-MS/MS assay: All samples showed no detectable levels with assay sensitivity around 20 pg/mL
  - ELISA assay: All samples showed detectable levels, with most >200 pg/ml using a commercially available ELISA kit.

- What the parallelism data look like if the assay lacks specificity?

Parallelism and Specificity

Sample dilution	Human Urine Lot											
	ELISA measured concentration after dilution, pg/mL											
	Normal 1	Normal 2	Normal 3	Asthma 1	Asthma 2	Asthma 3	Asthma 4	Asthma 5	Asthma 6	Asthma 7	Asthma 8	Asthma 9
2	225.8	120.9	205.8	233.1	AQL	AQL	AQL	20.3	1407.4	209.8	AQL	514.4
4	176.8	124.9	165.4	180.7	AQL	AQL	AQL	19.3	905.3	150.9	865.4	366.8
8	166.4	72.4	160.6	191.3	1589.9	AQL	795.9	BQL	649.6	121.9	879.1	377.3



- Parallelism data is an indicator of relative accuracy
- Lack of parallelism after surrogate matrix optimization suggests potential specificity issues
- However parallelism does not guarantee specificity

Acknowledgements

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