DMPK Biologics

The use of parallelism to define biomarker assay parameters: a case study

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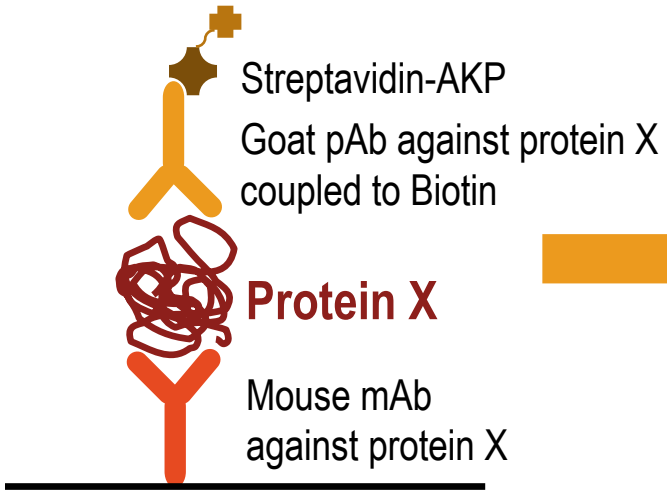
Measuring «protein x» in human serum



Assay kit recommended range:

0.06 – 6 ng/mL

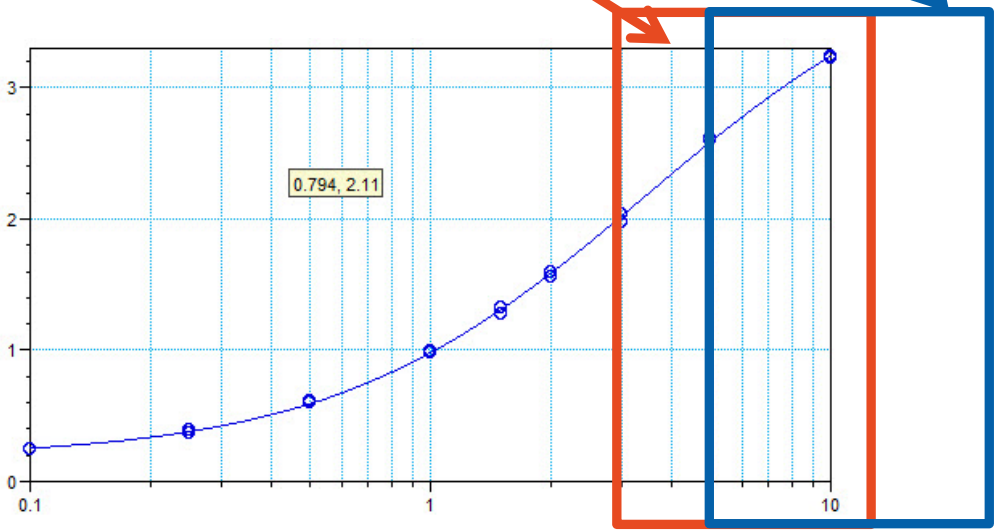
Recombinant «protein x» stock:

200 ng/mL

Healthy:
3-18 ng/mL
(n=45)

Diseased:
5-33 ng/mL
(n=33)

High Endogenous Values



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How do we define the assay range?

Does biomarker assay range need to be set based on spiked QCs as we do with PK assays?

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How would you prepare your QCs?

A. Prepare in human serum pool: dilute endogenous and/or spike to recombinant analyte at the desired levels.

B. Prepare in surrogate matrix: spike recombinant analyte at the desired levels.

C. Prepare in matrix depleted from analyte: spike recombinant analyte at the desired levels.

D. Use 2 or 3 individual serum with different endogenous levels.

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Agenda

- Publication Stevenson et al. 2014
- Why perform parallelism assessment?
- Using parallelism assessment to set assay parameters
- How validation was performed
- How sample analysis was performed

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An interesting alternative approach to set assay parameters

Publication by Stevenson et al. 2014

Bioanalysis. 2014 Jan;6(2):185-98. doi: 10.4155/bio.13.292.

Parallelism: considerations for the development, validation and implementation of PK and biomarker ligand-binding assays.

Stevenson LF[1], Purushothama S.

Author information

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Why performing parallelism for biomarker assays?

Publication by Stevenson et al. 2014

- To demonstrate that the (endogenous analyte) sample dilution-response curve is parallel to the standard concentration-response curve.
- To set the minimum required dilution (MRD)
- To set the LLOQ (estimation of assay sensitivity with respect to endogenous analyte)
- To assess selectivity

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Using parallelism to set MRD

Principle from Stevenson and al. 2014

Tested: 14 ind. sera (Healthy/Diseased)

- Dilution (2 to 1000)

Limits

- ALQ = If O.D. value sera > O.D value highest STD
- BLQ = If O.D. value sera < O.D value lowest STD

Reported: dilution adjusted concentration

- Adjusted result = Mean result x Dilution factor
- MRD at which all individual samples measures in assay range and multiple dilutions beyond demonstrate acceptable accuracy

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Using parallelism to set MRD

In healthy sera & diseased sera

Sample Dilution	Dilution-adjusted concentration (ng/mL; % nominal)																													
	1		2		3		4		5		6		7		8		9		10		11		12		13		14	
1:2	5.81	*(66)*	5.85	*(62)*	4.19	(88)	4.78	(85)	2.59	(79)	2.13	*(68)*	2.21	*(61)*	ALQ		ALQ		ALQ		ALQ		ALQ		ALQ		5.99	(83)
1:4	7.62	(87)	8.14	(87)	4.42	(93)	5.54	(98)	3.17	(97)	2.82	(91)	3.41	(95)	ALQ		ALQ		17.42	*(58)*	ALQ		18.66	(70)	ALQ		7.12	(98)
1:8*	8.74	(100)*	9.39	(100)*	4.77	(100)*	5.64	(100)*	3.28	(100)*	3.11	(100)*	3.6	(100)*	35.69	(100)*	25.88	(100)*	30.11	(100)*	31.62	(100)*	26.55	(100)*	34.28	(100)*	7.23	(100)*
1:10	8.78	(100)	8.87	(94)	4.58	(96)	5.61	(99)	2.94	(90)	3.04	(98)	3.31	(92)	37.71	(106)	25.33	(98)	34.16	(113)	35.77	(113)	27.06	(102)	38.81	(113)	6.65	(92)
1:20	9.31	(107)	9.00	(96)	3.65	(77)	4.83	(86)	2.94	(90)	3.25	(105)	2.62	(73)	40.78	(114)	27.53	(106)	38.13	(127)	36.54	(116)	29.84	(112)	41.4	(121)	6.34	(88)
1:50	7.22	(83)	6.75	(72)	BLQ		3.33	*(59)*	BLQ		BLQ		BLQ		32.67	(92)	23.11	(90)	32.83	(109)	30.22	(96)	25.98	(98)	35.61	(104)	4.03	*(56)*
1:100	BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		25.52	(72)	16.79	*(65)*	27.75	(92)	22.83	(72)	21.14	(80)	27.91	(81)	BLQ	
1:200	BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		18.89	*(53)*	BLQ		19.94	*(66)*	15.41	*(49)*	15.61	*(59)*	19.57	*(57)*	BLQ	
1:500	BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		BLQ	
1:1000	BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		BLQ		BLQ	

Red = If %nominal <70% and %nominal >130% // 13 out

= healthy sera = diseased sera

***Indicates chosen minimum required dilution where all samples measure in assay range and multiple dilutions beyond the minimum required dilution yield accurate results**

MRD Selected = 1:8

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Using parallelism to set LLOQ

Principle from Stevenson et al. 2014

Tested: 14 ind. sera (Healthy/Diseased)

- Dilution (8 to 1000)

Limits

- ALQ = If O.D. value sera > O.D value highest STD
- BLQ = If O.D. value sera < O.D value lowest STD

Reported: measured concentration

- Measured Concentration (Mean result)
- **Approach 1:** LLOQ=highest conc. observed at dilution where all samples returned accurate results
- **Approach 2:** LLOQ=highest conc. among the lowest conc. from each sera that were accurate

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Using parallelism to set LLOQ (Approach 1)

Common dilution method

Sample Dilution	Measured concentration (ng/mL)													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1:8	1.09	1.17	0.60	0.71	0.41	0.39	0.45	4.46	3.24	3.76	3.95	3.32	4.29	0.90
1:10	0.88	0.89	0.46	0.56	0.29	0.30	0.33	3.77	2.53	3.42	3.58	2.71	3.88	0.67
1:20*	0.47*	0.45*	0.18*	0.24*	0.15*	0.16*	0.13*	2.04*	1.38*	1.91*	1.83*	1.49*	**2.07***	0.32*
1:50	0.14	0.14	BLQ	0.07	BLQ	BLQ	BLQ	0.65	0.46	0.66	0.60	0.52	0.71	0.08
1:100	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	0.26	0.17	0.28	0.23	0.21	0.28	BLQ
1:200	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	0.09	BLQ	0.10	0.08	0.08	0.10	BLQ
1:500	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
1:1000	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ

Red = If %nominal <70% and %nominal >130%

= healthy sera = diseased sera

***Indicates the greatest dilution (1:20) at which all samples returned accurate results. Concentrations at MRD (1:8) were used as nominal. Bold: The highest concentration observed at that dilution is then set as the LLOQ**

LLOQ = 2.07 ng/mL

Conclusion: this approach **not optimal** if different subject populations with different baseline levels

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Using parallelism to set LLOQ (Approach 2)

Common concentration method

Sample Dilution	Measured concentration (ng/mL)													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1:8	1.09	1.17	0.60	0.71	0.41	0.39	0.45	4.46	3.24	3.76	3.95	3.32	4.29	0.90
1:10	0.88	0.89	0.46	0.56	0.29	0.30	0.33	3.77	2.53	3.42	3.58	2.71	3.88	0.67
1:20	0.47	0.45	0.18*	0.24*	0.15*	0.16*	0.13*	2.04	1.38	1.91	1.83	1.49	2.07	0.32*
1:50	0.14*	0.14*	BLQ	**0.07**	BLQ	BLQ	BLQ	0.65	**0.46***	0.66	0.60	0.52	0.71	**0.08**
1:100	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	0.26*	**0.17**	0.28*	0.23*	0.21*	0.28*	BLQ
1:200	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	**0.09**	BLQ	**0.10**	**0.08**	**0.08**	**0.10**	BLQ
1:500	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
1:1000	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ

Red = If %nominal <70% and %nominal >130%

= healthy sera = diseased sera

***Indicates the greatest dilution for each individual sample that returned accurate results. Concentrations at MRD (1:8) were used as nominal. Bold: All samples measured accurately at or below 0.46 ng/mL regardless of dilution factor, so the common concentration that was accurate for all samples was 0.46 ng/mL**

LLOQ Selected = 0.25 ng/mL

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About Selectivity…

«The same or similar experiment may also be used as selectivity assessment, since a demonstration of parallelism across multiple individuals effectively demonstrates that the endogenous analyte is being selectively measured in the context of complex matrix components»

Conclusion: No spike-recovery experiment in individual sera was done but selectivity was assessed based on parallelism.

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Selected parameters during Assay development

- *Minimum Required Dilution: 8*
- *LLOQ: 0.25 ng/mL (2 ng/mL in 100% serum)*
- *Selectivity demonstrated using parallelism with 14 individual sera*
- *Calibrators in assay buffer: 0.1, 0.25, 0.5, 1, 1.5, 2, 3, 5, 10 ng/mL*

QC Levels	Levels (100% matrix) (ng/mL)	Levels (MRD 8) (ng/mL)
QC1 (LLOQ)	2	0.25
QC2	6	0.75
QC3	10	1.25
QC4	20	2.50
QC5 (ULOQ)	40	5.00

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How validation was performed

Validation Parameters	QCs in Surrogate Matrix (recombinant analyte)	QCs using human sera (endogenous analyte)
Precision & Accuracy	5 levels spiked (2, 6, 10, 20 & 40 ng/mL) in bulk in 100% surrogate -> frozen	2 human sera at conc. ~LLOQ after dilution (~0.25 ng/mL) 2 human sera diluted at MRD (100% serum aliquoted & frozen)
Parallelism	N.A.	4 human sera diluted (8x-128x)
Stability (short & 2 years long term)	2 levels spiked (6 & 20 ng/mL (Bulk + frozen)	2 human sera (100% serum, aliquoted + frozen)

"Other parameters assessed as well: Specificity (interference), hook effect, ISR (study specimen)"

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How sample analysis was performed

- Calibration Curve prepared in assay buffer
- Using only QCs (3 levels) prepared in surrogate matrix (bulk preparation + frozen)
- MRD applied to QCs in surrogate matrix and specimens

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Conclusion

→The use of endogenous analyte facilitated assay development by setting assay parameters that were successfully validated

→Assay for protein x was very robust during sample analysis and allowed quantification in the required range

→Do not try to squeeze biomarker assay into PK principles

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Thank you

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