If the shoe doesn't fit, must we change the shoe?

Managing expectations around using 'off the shelf' biomarker validations

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CoU Strategy - Biomarkers and beyond...

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Background

For a long period, our lab did not have a policy of maintaining a portfolio of ‘validated’ biomarker assays (although we often got asked for our ‘biomarker list’...and still do)

When the lab first purchased a Simoa HDX instrument in 2020 we made the decision to perform an in-house validation of a commonly-requested neurological biomarker (BMx)

Validation of any biomarker assay was performed in line with our existing biomarker SOP, which, while a distinct document to the PK SOP, did apply BMV principles

What happens when a Sponsor wants to use the assay?

Resolian: Issue a questionnaire

- What is the purpose of measuring the biomarker(s)?
- Is the study exploratory, pivotal data that will be cited or a clinical end point
- Do you know the expected range of sample concentrations for the population you will be measuring?
- Is the Biomarker expected to be up or down regulated and by what extent?

- Scope
- Context of use
- Analyte, biology, and info on biological variation
- Assay requirements and specifications
- Timelines/planning

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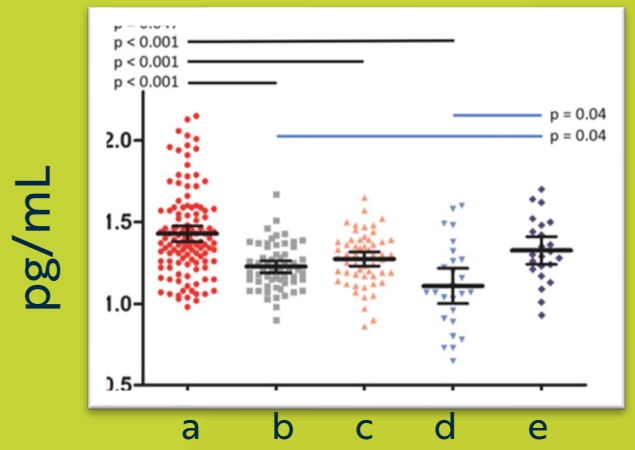
Intended use of the data

- Biomarker of neurodegeneration – understanding patient population

[CSF] ↑ [serum] ↓↓



a – neuro 1
b - healthy
c – neuro 2
d – target pop 1
e – target pop 2

- Report data as % change vs baseline
Sample analysis at study end: all samples will be analysed in batches at end of each group

FPFV 2020

LPLV late 2023

Validating the assay following the principals of the PK BMV resulted in limitations with its utility

Precision: very small changes in analyte concentration can be biologically relevant. The actual performance of the assay was very tight, however acceptance criteria was based on PK in the legacy validation

CSF samples: The original validation was performed in serum and plasma

Lot-to-lot bridging of kit: The Simoa kit has a shelf-life of 1 year (not possible to run kits beyond this), internal validation data was inadequate; lack of manufacturer data

Evolving the ‘validated’ assay to be fit for its context of use

Precision: employ new strategy using performance-based acceptance criteria, which links the precision of the method to the accuracy acceptance criteria

- No additional wet work, the dataset can be reanalysed to set acceptance limits based off the StDev of the precision data for each QC, providing original QCs are still available and within stability

CSF samples: Parallelism in CSF was performed and LTS samples were laid down asap

Lot-to-lot bridging of kit: A little trickier to resolve…

Reported validation data on lot-to-lot

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- 5 QC samples
- 2 test lots (502186 / 501998) tested once on 2 different days (head-to-head with reference)
- Reference lot (502183) tested twice (on each day)

Day 1

Ref

QC performance against Original Kit lot (502183)

Run Date	Curve Number	LLOQ 2.34 pg/mL	%RE	LQC 4.22 pg/mL	%RE	MQC 5.68 pg/mL	%RE	HQC 33.6 pg/mL	%RE	ULOQ 124 pg/mL	%RE
26-Apr-2020	24	2.63	12.4	4.73	12.1	6.47	13.9	33.7	0.3	130	4.8
		2.30	-1.7	4.51	6.9	6.26	10.2	35.4	5.4	139	12.1
		2.22	-5.1	4.59	8.8	6.39	12.5	35.3	5.1	130	4.8
Intrarun Mean		2.38		4.61		6.37		34.8		133	
Intrarun SD		0.217		0.111		0.106		0.954		5.20	
Intrarun %CV		9.1		2.4		1.7		2.7		3.9	
Intrarun %RE		1.7		9.2		12.1		3.6		7.3	
n		3		3		3		3		3	

Test

QC performance against additional Kit lot (502186)

Run Date	Curve Number	LLOQ 2.34 pg/mL	%RE	LQC 4.22 pg/mL	%RE	MQC 5.68 pg/mL	%RE	HQC 33.6 pg/mL	%RE	ULOQ 124 pg/mL	%RE
26-Apr-2020	26	2.89	23.5	5.07	20.1	6.86	20.8	34.1	1.5	127	2.4
		2.54	8.5	4.84	14.7	6.64	16.9	35.8	6.5	136	9.7
		2.46	5.1	4.93	16.8	6.77	19.2	35.7	6.3	128	3.2
Intrarun Mean		2.63		4.95		6.76		35.2		130	
Intrarun SD		0.229		0.116		0.111		0.954		4.93	
Intrarun %CV		8.7		2.3		1.6		2.7		3.8	
Intrarun %RE		12.4		17.3		19.0		4.8		4.8	
n		3		3		3		3		3	

Day 2

Ref

QC performance against Original Kit lot (502183)

Run Date	Curve Number	LLOQ 2.34 pg/mL	%RE	LQC 4.22 pg/mL	%RE	MQC 5.68 pg/mL	%RE	HQC 33.6 pg/mL	%RE	ULOQ 124 pg/mL	%RE
28-Apr-2020	30	2.03	-13.2	4.05	-4.0	5.72	0.7	32.3	-3.9	119	-4.0
		2.07	-11.5	3.99	-5.5	4.91	-13.6	31.0	-7.7	125	0.8
		1.98	-15.4	4.01	-5.0	5.52	-2.8	31.1	-7.4	120	-3.2
Intrarun Mean		2.03		4.02		5.38		31.5		121	
Intrarun SD		0.0451		0.0306		0.422		0.723		3.21	
Intrarun %CV		2.2		0.8		7.8		2.3		2.7	
Intrarun %RE		-13.2		-4.7		-5.3		-6.3		-2.4	
n		3		3		3		3		3	

Test

QC performance against additional kit lot (501998)

Run Date	Curve Number	LLOQ 2.34 pg/mL	%RE	LQC 4.22 pg/mL	%RE	MQC 5.68 pg/mL	%RE	HQC 33.6 pg/mL	%RE	ULOQ 124 pg/mL	%RE
28-Apr-2020	32	2.32*	-0.9	4.26*	0.9	5.85	3.0	31.6	-6.0	116	-6.5
		2.35*	0.4	4.19*	-0.7	5.08	-10.6	30.3	-9.8	123	-0.8
		2.27*	-3.0	4.21*	-0.2	5.66	-0.4	30.5	-9.2	118	-4.8
Intrarun Mean		2.31*		4.22*		5.53		30.8		119	
Intrarun SD		0.0404		0.0361		0.401		0.700		3.61	
Intrarun %CV		1.7		0.9		7.3		2.3		3.0	
Intrarun %RE		-1.3		0.0		-2.6		-8.3		-4.0	
n		3		3		3		3		3	

Precision runs from a single kit lot - max %CV 9.6 (inter) 6.4 (intra)

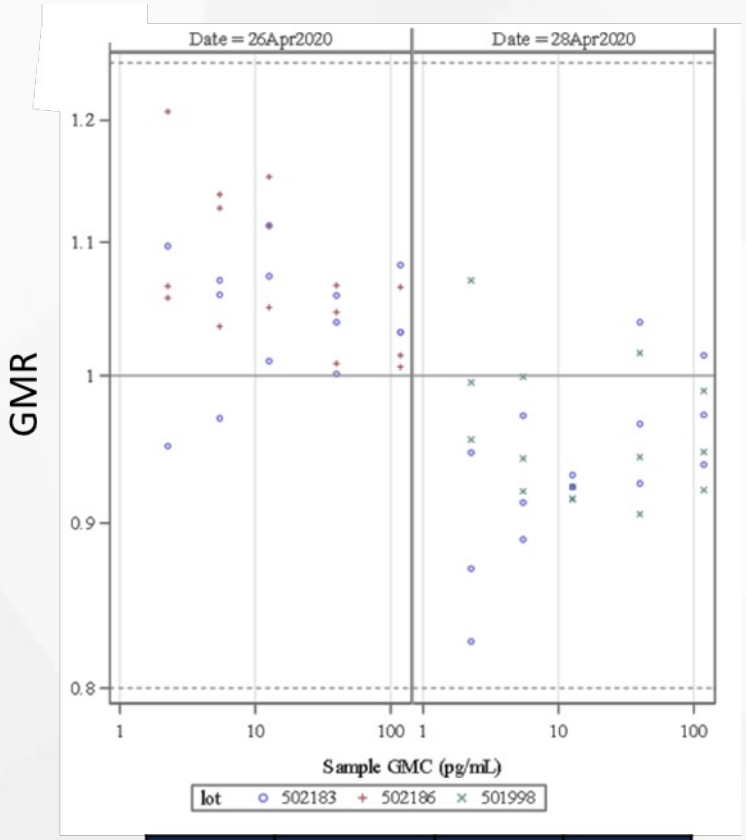
Existing validation: re-evaluating the analytical variability

				Run	QC1	QC2	QC3	QC4	QC5
Plasma	Lot to Lot	502183	26-avr-20	24	2,63	4,73	6,47	33,7	130
Plasma	Lot to Lot	502183	26-avr-20	24	2,3	4,51	6,26	35,4	139
Plasma	Lot to Lot	502183	26-avr-20	24	2,22	4,59	6,39	35,3	130
Plasma	Lot to Lot	502186	26-avr-20	26	2,89	5,07	6,86	34,1	127
Plasma	Lot to Lot	502186	26-avr-20	26	2,54	4,84	6,64	35,8	136
Plasma	Lot to Lot	502186	26-avr-20	26	2,46	4,93	6,77	35,7	128
Plasma	Lot to Lot	502183	28-avr-20	30	2,03	4,05	5,72	32,3	119
Plasma	Lot to Lot	502183	28-avr-20	30	2,07	3,99	4,91	31	125
Plasma	Lot to Lot	502183	28-avr-20	30	1,98	4,01	5,52	31,1	120
Plasma	Lot to Lot	501998	28-avr-20	32	2,32	4,26	5,85	31,6	116
Plasma	Lot to Lot	501998	28-avr-20	32	2,35	4,19	5,08	30,3	123
Plasma	Lot to Lot	501998	28-avr-20	32	2,27	4,21	5,66	30,5	118

Reference lot

Test lot 1

Test lot 2



	Ref QC1 conc	Mean	%CV
Day 1	2.63	2.21	10.9
	2.30		
	2.22		
Day 2	2.03		
	2.07		
	1.98		

A strong day effect is observed for the reference lot when the data is graphed, which isn't obvious when calculating %CV

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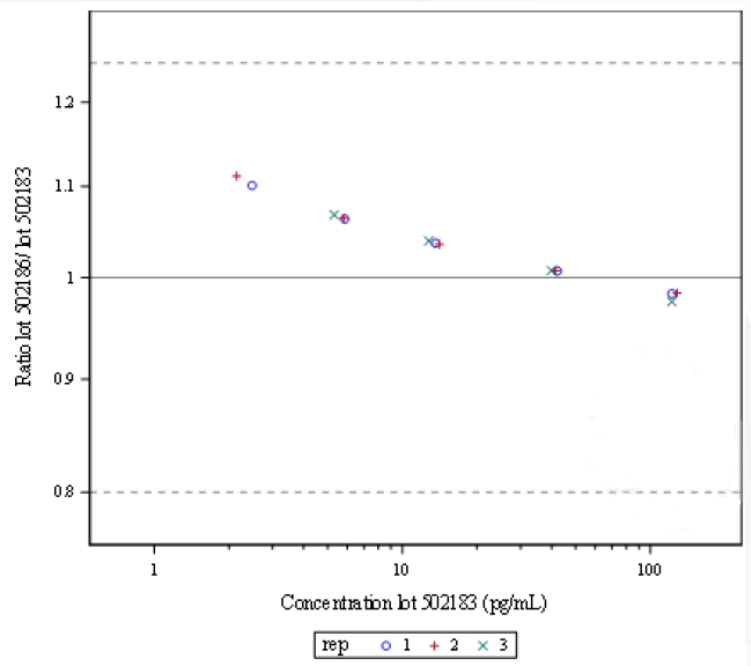
Plasma	Lot to Lot	501998	28-avr-20	32	2,27	4,21	5,66	30,5	118

Reference lot

Test lot 1

Test lot 2

Lot 502186 vs lot 502183			95% CI	
Matrix	N	GMR	Lower	Upper
Plasma	15	**1.04**	1.02	1.07
Serum	14	**1.03**	1.01	1.06



- Overall GMR is close to 1
- BUT trend over the analytical range evident

Emphasises the importance of visualising data over concentration values

What happens when a Sponsor wants to use the assay, but not the validation?

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- The N is limited: limit statistical confidence in outcome (reliability)
- The design is not balanced, hampering statistical analysis (confounding day and kit effect)
- A bias in the lower part of the curve is seen, although small, the potential biological effect is also small.

Was our ‘validated’ assay fit for purpose?

- Taken together these factors meant that the existing validation did not meet the intended context of use

Evolving the ‘validated’ assay to be fit for its context of use

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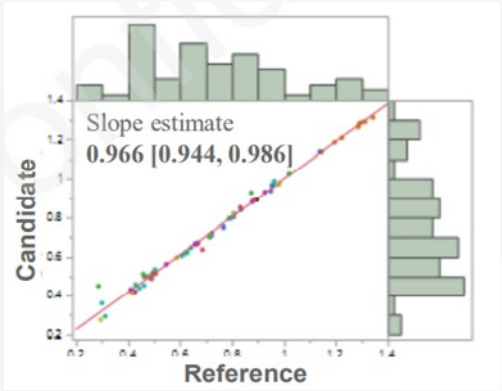
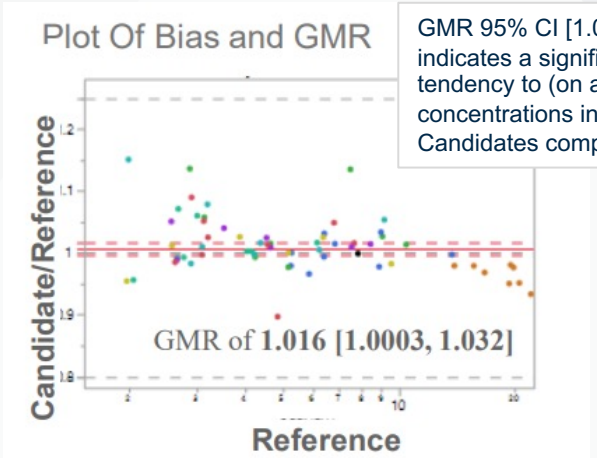
Kit lot bridging: experimental design and testing strategy

- Use an equivalence approach
  - ➢Does not penalise more precise assays, larger sample panels or ranges
- Bland-Altman geometric mean of ratios (GMR) approach and Deming regression recommended for evaluation of the comparability





- The new lot is considered equivalent to the old lot if the outputs of the regression line falls within predefined acceptance criteria, which should be based on the COU and analytical performance.

Kit lot bridging: practical considerations

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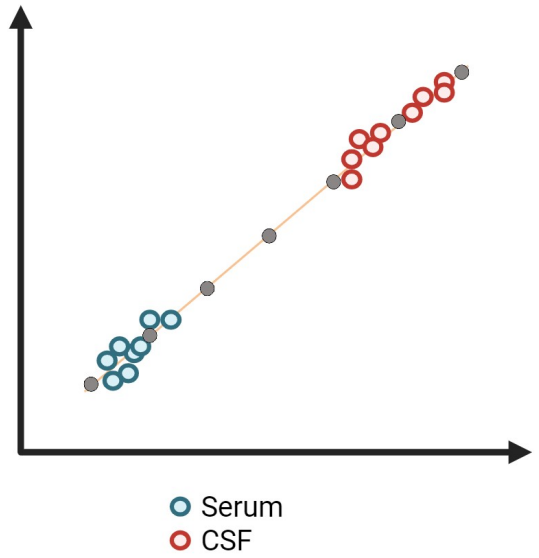
- A bridge panel, consisting of 30-40 individual samples, concentrations that span the analytical range
- Side-by-side testing of the new and current lot (1 plate with REF and 1 plate with Candidate) on the same day by the same operator
- Repeated testing of the bridge panel over multiple separate days
- How do you distribution of samples over the analytical range when your biomarker is at low endogenous levels in serum?
  - CSF spiked serum samples



Take 15-20 individual CSF samples
Screen endogenous analyte concentrations in CSF in one run

↓

Select 10 with biggest span of concentrations

↓

Take individual serum samples and spike each at 2 levels with CSF to create a panel of 30 serum samples

Technology Specific Considerations

What limitations or specific considerations does the technology dictate?

Sample volume

- **2 mL per sample, per bridge, considerable sample volume required on platform, plus the additional need to perform this potentially on a number of occasions in order to cover sample analysis**
- **Do you keep the same panel for consecutive bridging experiments? Do you have stability to do that?**

Can’t take advantage of instrument capacity

- **New lot and old lot run as separate experiments (i.e. not multi-plate)**

Can take advantage of automated property

- **No other inter-run or intra-day variable to take into account (analyst negligible, reagent prep no relevant)**

Final Points

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- Kit lot bridging takes a lot of planning, and consideration of the most appropriate statistical considerations in order for it to be FFP
- Need to make sure an appropriate LTS is on-going to enable the bridging panel to be stored and not generated each time bridging is required
- In other circumstances, QCs might suffice, depending on CoU, or simply trending to ensure that EQC variability remains within the limits of trending analysis
- Keep asking manufacturers to better evaluate the *true* shelf life of their reagents

- Under the principals of CoU, we can't have requests for the 'biomarker list' anymore.

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- Under the principals of CoU, we can't have requests for the 'biomarker list' anymore.
- Kindly refrain from asking for a list of validated biomarker assays.

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