

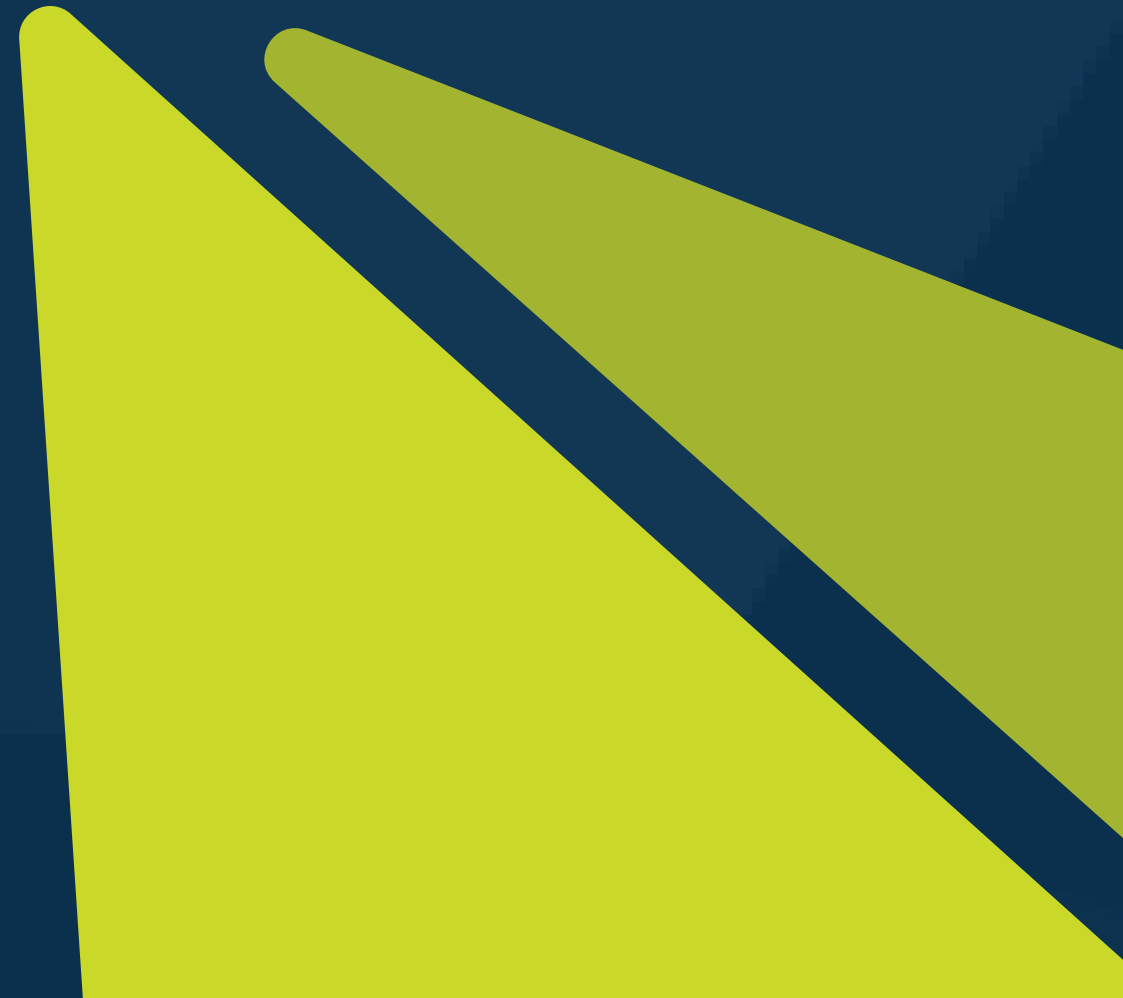
If the shoe doesn't fit, must we change the shoe?

Managing expectations
around using 'off the shelf'
biomarker validations

16 November 2023

CoU Strategy - Biomarkers and beyond...

Richard Hughes, Resolian, Cambridge UK



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?

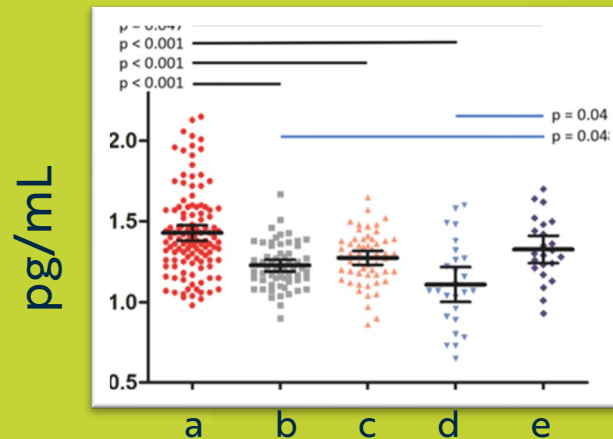


- Scope
- Context of use
- Analyte, biology, and info on biological variation
- Assay requirements and specifications
- Timelines/planning

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

CHALLENGE



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

Long-term stability of 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)
Note that Quanterix assures that the kit is highly reproducible and that many of their customers do not bridge kit lots, but no data has been shared so far to support this



SOLUTION

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

Long-term stability of 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

- 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

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 |
|---------------|--------------|--------------------|------|-------------------|------|-------------------|------|-------------------|-----|-------------------|------|
| 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 |
| Intraran Mean | | 2.38 | | 4.61 | | 6.37 | | 34.8 | | 133 | |
| Intraran SD | | 0.217 | | 0.111 | | 0.106 | | 0.954 | | 5.20 | |
| Intraran %CV | | 9.1 | | 2.4 | | 1.7 | | 2.7 | | 3.9 | |
| Intraran %RE | | 1.7 | | 9.2 | | 12.1 | | 3.6 | | 7.3 | |
| n | | 3 | | 3 | | 3 | | 3 | | 3 | |

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 |
| Intraran Mean | | 2.03 | | 4.02 | | 5.38 | | 31.5 | | 121 | |
| Intraran SD | | 0.0451 | | 0.0306 | | 0.422 | | 0.723 | | 3.21 | |
| Intraran %CV | | 2.2 | | 0.8 | | 7.8 | | 2.3 | | 2.7 | |
| Intraran %RE | | -13.2 | | -4.7 | | -5.3 | | -6.3 | | -2.4 | |
| 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 |
| Intraran Mean | | 2.63 | | 4.95 | | 6.76 | | 35.2 | | 130 | |
| Intraran SD | | 0.229 | | 0.116 | | 0.111 | | 0.954 | | 4.93 | |
| Intraran %CV | | 8.7 | | 2.3 | | 1.6 | | 2.7 | | 3.8 | |
| Intraran %RE | | 12.4 | | 17.3 | | 19.0 | | 4.8 | | 4.8 | |
| n | | 3 | | 3 | | 3 | | 3 | | 3 | |

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 |
| Intraran Mean | | 2.31* | | 4.22* | | 5.53 | | 30.8 | | 119 | |
| Intraran SD | | 0.0404 | | 0.0361 | | 0.401 | | 0.700 | | 3.61 | |
| Intraran %CV | | 1.7 | | 0.9 | | 7.3 | | 2.3 | | 3.0 | |
| Intraran %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)

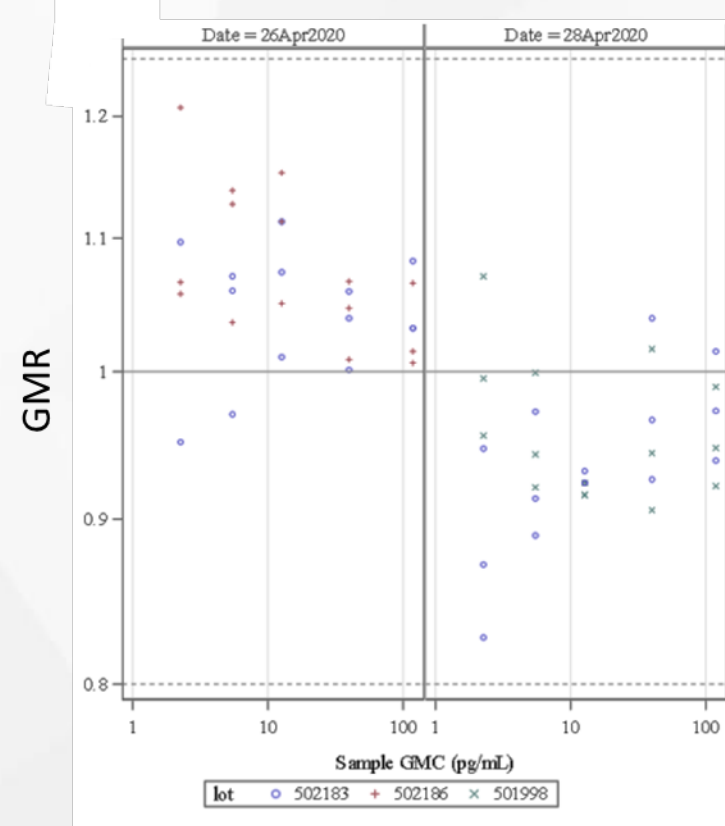
Existing validation: re-evaluating the analytical variability

| | | | | 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



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

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

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

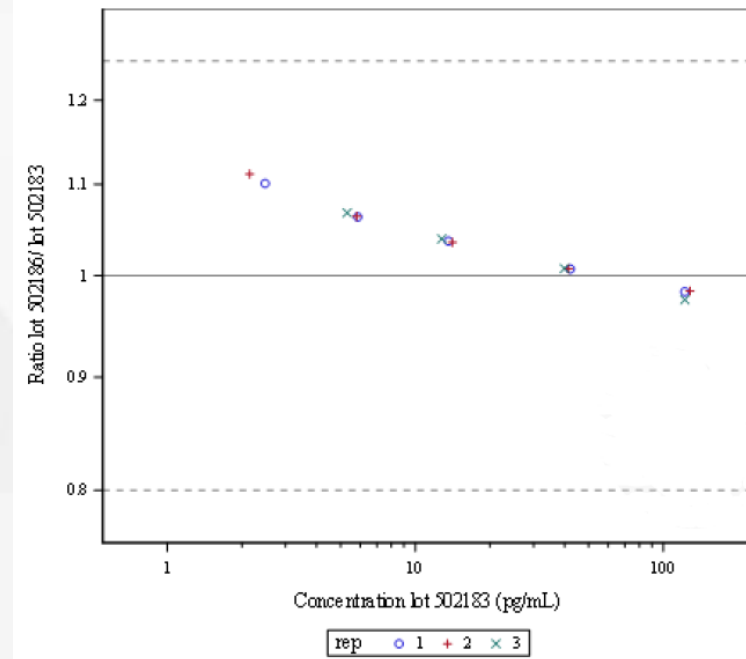
Test lot 1

Test lot 2

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

Emphasises the importance of visualising data over concentration values

| 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 |





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

- 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.

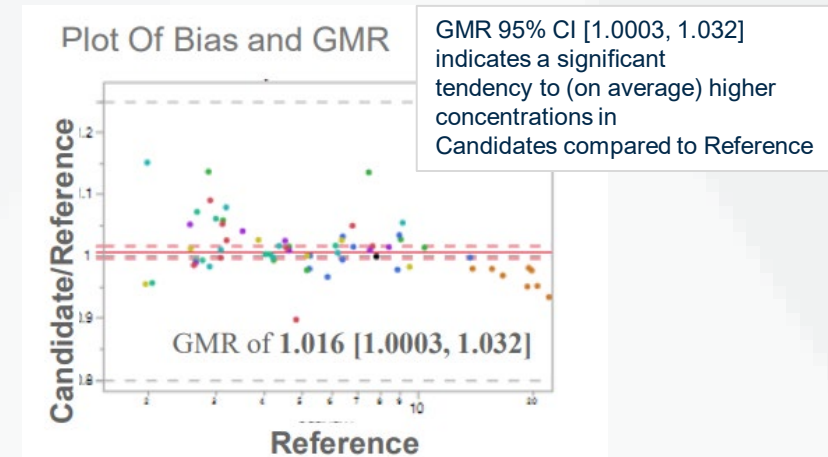
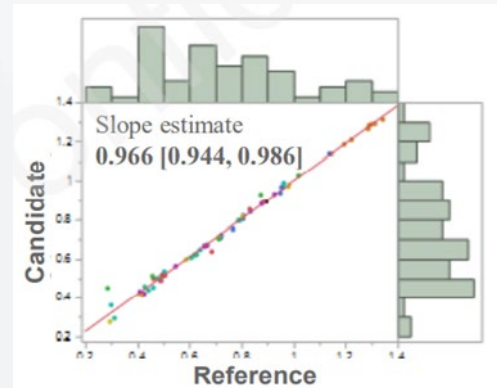
Was our 'validated' assay fit for purpose?

- 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

Kit lot bridging: experimental design and testing strategy

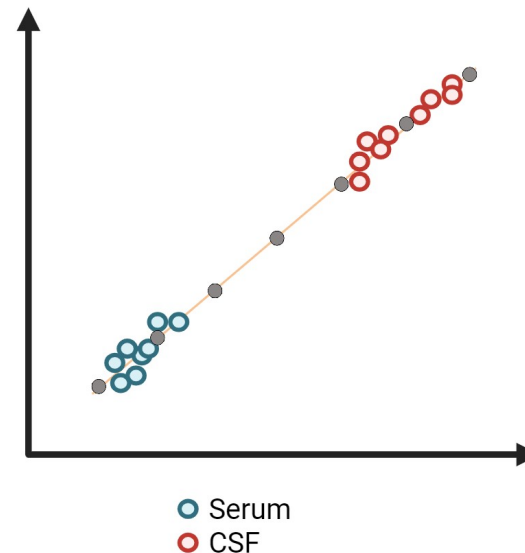
- Use an equivalence approach
 - Does not penalise more precise assays, larger sample panels or ranges
- Bland-Altman geometric mean of ratios (GMR) approach and Deming regression recommended for evaluation of the comparability



- 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

- 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 plasma/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 (pre-screened) 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

- 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.

Final Points

- 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.
- Please stop asking me for a list of validated assays.

RESOLIAN



Proven expertise.
Worldwide access.