Assay Cross Validation

Recent experiences in transferring bioanalytical assays from sponsor to CRO partners and between CROs

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Assay Cross Validation Between Bioanalytical Labs

A Journey of discovery…

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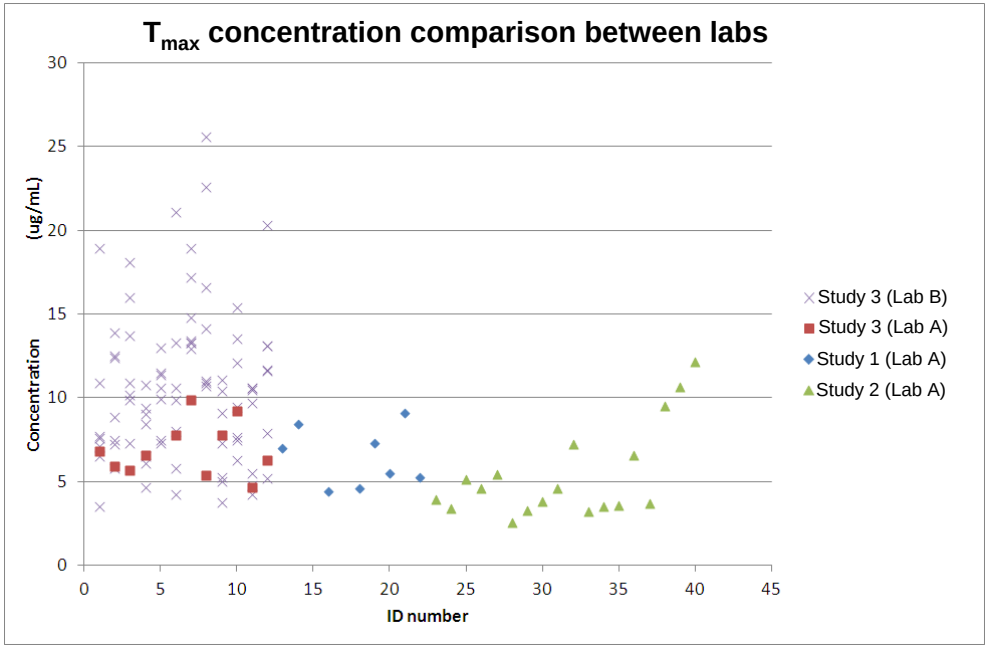
A Journey of Discovery…

1. The beginning - Questions raised by clinical customers
2. The destination
3. Navigating the way
4. The road so far
5. Where to next?
6. “Stop and Ask for Directions”
7. “Are we nearly there yet”?
8. Acknowledgements

The Beginning…

Questions raised by clinical customers (example 1)

Study	Lab	Dose (mg/kg)	Cmax (ng/mL)	Tmax (hr)	AUC[0-24hr] hr*µg/mL
1	A	6	6238	3.08	57.3
2	A	6	4852	2.57	45.54
3	**B**	**6**	**8703**	**3.99**	**103.4**



- "Any explanation for between study differences in analyte PK?"
- Differences observed when bioanalysis conducted at two different laboratories
- "When you transferred the assay, what assurances did you have of assay/result equivalence?"
- Full validation at both labs but no cross-over or direct comparison

The Beginning…

Questions raised by clinical customers (example 2)

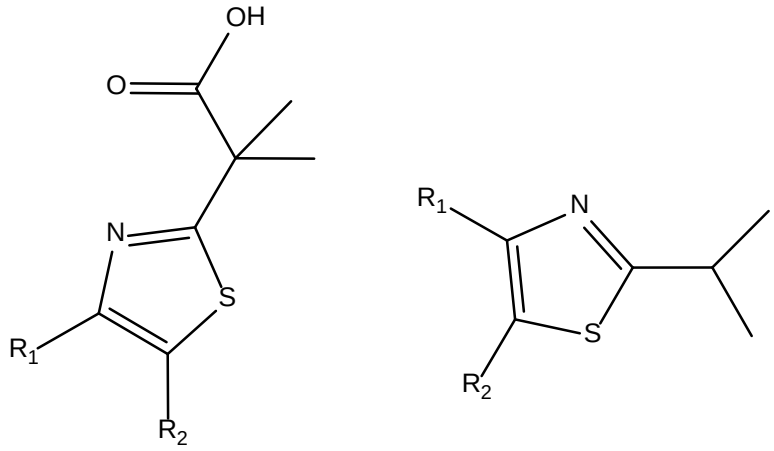
- Many previous clinical studies with in-house bioanalytical support
- 1st study in a new patient population
  - Bioanalysis supported at contract
- Higher exposure (v. close to safety margin) observed in some subjects
- Population specific or due to difference in bioanalytical facility?
- Retrospective cross-validation confirmed equivalence between both analytical facilities/methods

Study	AUC	C max	T max
1	406 (310 – 652)	23.5 (16 – 46)	6.5
2	286 (138 – 471)	17.1 (8.8 – 40)	6
3	312 (165 – 863)	18.3 (8.8 – 74)	4
4	315 (144 – 735)	17.5 (6.9 – 47.1)	6
5	570.9 (168 - 1873)	41.9 (8.12 – 133)	3

The Beginning…

Questions raised by clinical customers (example 3)

- Parent drug plus 5 metabolites
  - Multiple assays (protein ppt & liq/liq extraction)
- One of the metabolites (M4) was found to be unstable in acidic conditions
- Assay validation went well, both in-house and at contract
- Higher than expected levels of (M8) metabolite observed during study support at contract
- Root cause identified as difference in supplier of ethyl acetate (for liq/liq extraction)
  - Lower pH (1 unit) of affected batch caused degradation of a labile metabolite
  - Presence of acetic acid impurity



M4 → M8

M4 concentrations approx. 100x higher than M8

The Destination…

Conduct a formal “cross validation” as part of all method transfers

- When?
  - Transfer of a bioanalytical assay from GSK to CRO
  - Transfer of a bioanalytical assay (for a GSK asset) between CROs
  - As an additional experiment on top of a full (e.g. 3-run) assay validation

- How?
  - Design a process for ensuring “assay equivalence” between bioanalytical labs (and assays)
  - Analyse a set of “test samples” at the originator and comparator labs
- Why?
  - Add confidence that any differences in PK data observed between studies is not attributable to the analytical assay or test facility

Navigating the Way…

Definition of test samples

- QCs
  - All assay validation concentrations?
  - Study levels only (e.g. low/mid/high)?
  - Include dilution QCs?

- Use of unknown study samples
  - Individual samples?
  - Pooled samples?
  - Informed consent!!!!
  - Human Tissues Act

The clinical ICF template contains language stating that samples may be used for further research

e.g. *samples may be used by GSK **or shared by GSK with other companies** or universities to better understand your disease, other diseases or conditions, **or to further develop the study drug or other drugs.***

*"Another kind of blood sample, called "PK" samples are also going to be taken in this study. PK blood samples are tested to see exactly how much study medicine is in your blood. This information tells us how long the drug stays in your blood. **Once the information is obtained from the PK samples, the samples are destroyed.** For the PK samples, your name is substituted with a number so the laboratory does not know who has given the samples. The only people who can match the number to your name are the doctor and his or her staff, and the other groups of people whose job it is to make sure that the study is being done right."*

"The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents"

Navigating the Way…

Definition of Acceptance Criteria

"surely this is what's important?"

- QCs
  - % difference between mean at each lab?
  - %CV requirement for replicates?
  - % difference from nominal?

"who cares if % difference is OK?"

Lab A = +8%
Lab B = +17%

Lab A = +11%
Lab B = -11%

"seems sensible"

- Unknown study samples…
  - 20 to 30 samples in singlet
  - 4-6 samples in replicate (say n=6)
  - ⅔ rds within ± 20% of mean?
  - All samples within ± 20% of mean?
  - Directional bias even if criteria pass

OK for pooled samples

OK for individual samples, but that's a lot of pools!

OK for large number of test samples, but what about 4-6 pools?

May be OK for 4-6 pools (tested in replicate)

Risk of acceptance criteria being prohibitive

Risk of acceptance criteria being too liberal

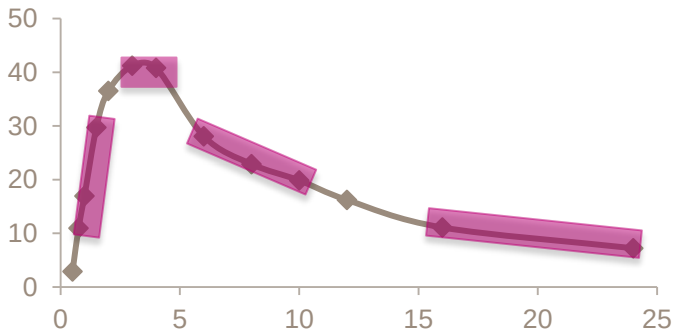
Navigating the Way

"so which way do we go?"

Navigating the Way…

Pick a road and follow it

- Set of “test samples” analysed at both labs in replicates (n=6)
  - 3 QCs (low, mid, high)
  - 3-4 incurred sample pools
  - If incurred samples unavailable then use 6-7 QC levels (≥ 3x LLOQ)



- close to C_{max}
- close to C_{min}
- between C_{max} & C_{min} (elimination phase)
- pre-C_{max} (absorption phase) - *may not be possible if T_{max} is short or for iv dose*
- Where profiles unavailable (e.g. trough sampling) – create 4 pools with a range of concentrations

- Acceptance criteria:
  - Precision ≤ 15% for each set of replicates (≤ 20% for ligand binding assays)
  - % difference (between each lab mean) within ± 20% for <u>all</u> test samples (± 30% for LBAs)

The Road So Far….

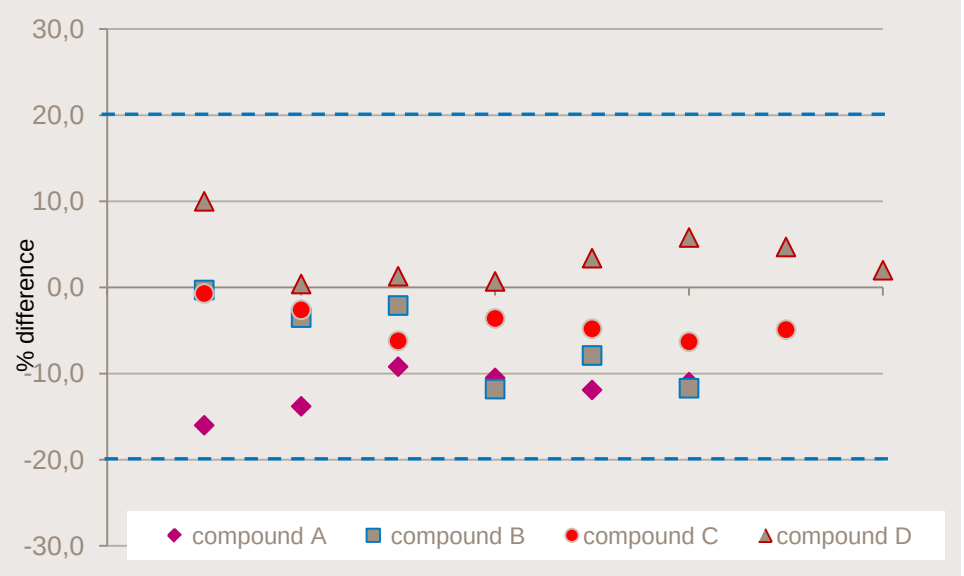
Example data sets since implementing this process

4 1 1

The Road So Far….

Examples when it went “well”: NCEs using LC-MS/MS

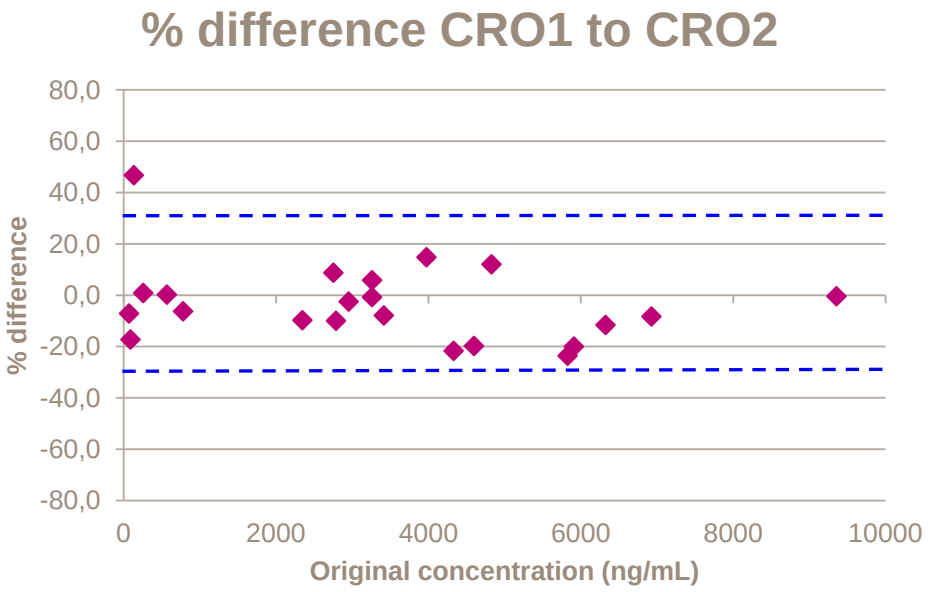
- Parent drug (A) + metaboite (B): GSK=> CRO
  - 6 test samples: 3x QC, 3x pooled samples
  - % difference range: -16% to -9% (parent), -12% to 0% (metab)
- Parent drug (C): GSK => CRO
  - 7 test samples: 3x QC, 4x pooled samples (including pool above HLQ)
  - % difference range: -6% to -1%
- Parent drug (D): CRO 1 => CRO 2
  - 8 test samples: 4x QC, 4x pooled samples (including QC & pool above HLQ)
  - % difference range: +1% to +10%

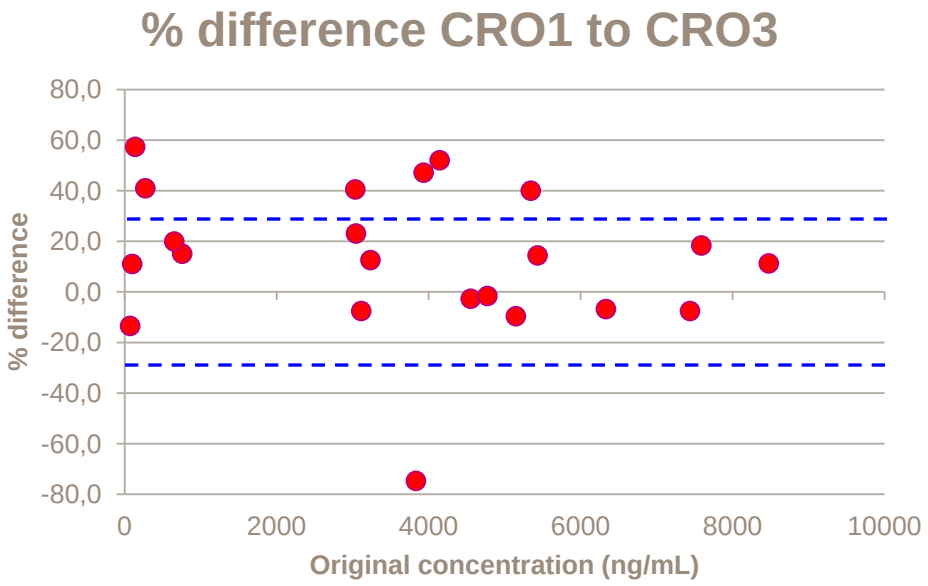


The Story So Far….

Examples when it went “well”: large molecule LBA

- Large molecule (E) **by LBA**: CRO1 => CRO2 **and** CRO 1 => CRO 3
  - 22 (quantifiable) test samples in singlet: > ⅔rds with % difference within 30%





CRO1 to CRO2:
1 of 22 > 30% difference
i.e. 4.5%

CRO1 to CRO3:
7 of 22 > 30% difference
i.e. 31.8%

The Road So Far…

Example with resolved issues (part 1)

- Parent + metabolite
  - Directional bias observed…

Originator Lab	Compound P (parent)				
	QC A	QC B	QC C	QC D	QC E
Mean	0.185	0.616	9.886	157.599	195.802
S.D.	0.017	0.015	0.272	1.970	2.755
%CV	9.0	2.4	2.7	1.3	1.4
% Bias	-7.8	2.6	-1.1	-1.5	-2.1
n	6	6	6	6	6

Comparator Lab	Compound P (parent)				
	QC A	QC B	QC C	QC D	QC E
Mean	0.239	0.740	12.093	189.911	231.816
S.D.	0.011	0.008	0.124	4.236	3.858
%CV	4.4	1.1	1.0	2.2	1.7
% Bias	19.3	23.3	20.9	18.7	15.9
n	6	6	6	6	6
% difference	-25.53	-18.35	-20.09	-18.60	-16.84

Originator Lab	Compound M (metabolite)				
	QC A	QC B	QC C	QC D	QC E
Mean	0.93	2.95	49.54	768.64	959.47
S.D.	0.05	0.05	1.02	19.83	12.47
%CV	5.2	1.8	2.1	2.6	1.3
% Bias	-7.5	-1.7	-0.9	-3.9	-4.1
n	6	6	6	6	6

Comparator Lab	Compound M (metabolite)				
	QC A	QC B	QC C	QC D	QC E
Mean	1.083	3.380	54.746	871.301	1079.689
S.D.	0.053	0.059	0.590	12.753	18.286
%CV	4.9	1.7	1.1	1.5	1.7
% Bias	8.3	12.7	9.5	8.9	8.0
n	6	6	6	6	6
% difference	-15.75	-13.59	-9.98	-12.52	-11.79

- Investigation followed
  - Including a bi-directional cross validation (i.e. QCs prepared by each lab and analysed by both)

The Road So Far…

Example with resolved issues (part 2)

- Differences in STD/QC spiking scheme identified as one possible contributing factor

Originator Lab	Compound P (parent)				
	QC A	QC B	QC C	QC D	QC E
Mean	0.211	0.653	10.688	167.810	209.801
S.D.	0.026	0.051	0.669	10.212	15.480
%CV	12.2	7.8	6.3	6.1	7.4
% Bias	5.7	8.9	6.9	4.9	4.9
n	18	18	18	18	18

Comparator Lab	Compound P (parent)				
	QC A	QC B	QC C	QC D	QC E
Mean	0.238	0.717	11.416	180.386	221.876
S.D.	0.010	0.016	0.137	2.265	2.733
%CV	4.2	2.2	1.2	1.3	1.2
% Bias	18.8	19.4	14.2	12.7	10.9
n	6	6	6	6	6
% difference	-11.7	-9.2	-6.6	-7.2	-5.6

Originator Lab	Compound M (metabolite)				
	QC A	QC B	QC C	QC D	QC E
Mean	0.98	3.06	51.07	790.07	919.16
S.D.	0.09	0.15	1.49	22.67	79.14
%CV	9.2	5.0	2.9	2.9	8.6
% Bias	-1.7	2.1	2.1	-1.2	-8.1
n	18	18	18	18	18

Comparator Lab	Compound M (metabolite)				
	QC A	QC B	QC C	QC D	QC E
Mean	1.06	3.23	52.12	788.44	984.88
S.D.	0.04	0.07	0.86	7.96	20.54
%CV	3.9	2.1	1.6	1.0	2.1
% Bias	6.4	7.6	4.2	-1.4	-1.5
n	6	6	6	6	6
% difference	-7.9	-5.2	-2.0	0.2	-6.9

- Directional bias still observed – but much improved

The Road So Far

Example with unresolved issues (part 1)

- Combo assay (low pg/mL LLOQ for both analytes)
  - Directional bias observed

Originator Lab	Compound X						
	TS 1	TS 2	TS 3	TS 4	TS 5	TS 6	TS 7
Mean	1487.4	182.2	9704.8	32.3	951.5	86.0	1678.1
S.D.	17.3	7.6	137.7	4.8	20.0	2.3	28.2
%CV	1.2	4.2	1.4	14.8	2.1	2.6	1.7
Bias	-7.0	-8.9	-3.0	7.5	-4.9	-14.0	-6.8
n	6	6	6	6	6	6	6

Comparator Lab	Compound X						
	TS 1	TS 2	TS 3	TS 4	TS 5	TS 6	TS 7
Mean	1790.0	214.0	11616.7	36.4	1120.0	106.7	1946.7
S.D.	8.9	1.9	172.2	1.7	11.0	2.4	31.4
%CV	0.5	0.9	1.5	4.6	1.0	2.3	1.6
Bias	11.9	7.0	16.2	21.3	12.0	6.7	8.1
n	6	6	6	6	6	6	6
% difference	**-18.5**	**-16.1**	**-17.9**	**-12.0**	**-16.3**	**-21.4**	**-14.8**

Originator Lab	Compound Y						
	TS 1	TS 2	TS 3	TS 4	TS 5	TS 6	TS 7
Mean	796.3	194.8	5232.5	30.7	509.5	93.3	909.1
S.D.	5.29	7.73	80.59	0.97	6.68	1.42	26.69
%CV	0.7	4.0	1.5	3.2	1.3	1.5	2.9
Bias	-0.5	-2.6	4.6	2.2	1.9	-6.7	1.0
n	6	6	6	6	6	6	6

Comparator Lab	Compound Y						
	TS 1	TS 2	TS 3	TS 4	TS 5	TS 6	TS 7
Mean	938.2	217.0	6038.3	37.5	593.5	111.2	1111.7
S.D.	14.66	4.20	224.09	1.33	13.19	2.64	33.71
%CV	1.6	1.9	3.7	3.6	2.2	2.4	3.0
Bias	17.3	8.5	20.8	24.9	18.7	11.2	23.5
n	6	6	6	6	6	6	6
% difference	**-16.4**	**-10.8**	**-14.3**	**-20.0**	**-15.2**	**-17.4**	**-20.0**

- Investigation followed
  - Pointed towards differences in working solutions

The Road So Far

Example with unresolved issues (part 2)

- Repeat of cross validation after investigation…

Originator Lab	Compound X						
	TS 1	TS 2	TS 3	TS 4	TS 5	TS 6	TS 7
Mean	950.8	1730.7	31.3	97.8	9521.2	1567.9	193.9
S.D.	27.6	53.3	3.7	1.9	284.4	33.5	5.8
%CV	2.9	3.1	11.8	1.9	3.0	2.1	3.0
Bias	-4.9	-3.8	4.2	-2.3	-4.8	-2.0	-3.1
n	6.0	6.0	6.0	6.0	6.0	6.0	6.0

Comparator Lab	Compound X						
	TS 1	TS 2	TS 3	TS 4	TS 5	TS 6	TS 7
Mean	1105.0	2018.3	33.6	111.7	10405.0	1761.7	227.2
S.D.	43.7	56.4	1.5	4.6	601.1	39.7	9.8
%CV	4.0	2.8	4.5	4.1	5.8	2.3	4.3
Bias	10.5	12.1	11.9	11.7	4.1	10.1	13.6
n	6.0	6.0	6.0	6.0	6.0	6.0	6.0
% difference	**-15.0**	**-15.3**	**-7.1**	**-13.3**	**-8.9**	**-11.6**	**-15.8**

Originator Lab	Compound Y						
	TS 1	TS 2	TS 3	TS 4	TS 5	TS 6	TS 7
Mean	491.6	874.0	30.4	95.7	4849.8	785.4	201.6
S.D.	14.9	24.7	1.1	7.0	179.8	14.8	8.3
%CV	3.0	2.8	3.8	7.3	3.7	1.9	4.1
Bias	-1.7	-2.9	1.2	-4.3	-3.0	-1.8	0.8
n	6.0	6.0	6.0	6.0	6.0	6.0	6.0

Comparator Lab	Compound Y						
	TS 1	TS 2	TS 3	TS 4	TS 5	TS 6	TS 7
Mean	589.5	1080.0	39.2	119.2	5501.7	902.7	237.8
S.D.	13.6	40.0	2.8	2.9	305.0	23.8	10.0
%CV	2.3	3.7	7.0	2.4	5.5	2.6	4.2
Bias	17.9	20.0	30.7	19.2	10.0	12.8	18.9
n	6.0	6.0	6.0	6.0	6.0	6.0	6.0
% difference	**-18.1**	**-21.1**	**-25.5**	**-21.8**	**-12.6**	**-13.9**	**-16.5**

- Directional bias still observed
  - Investigation continues

Where Next

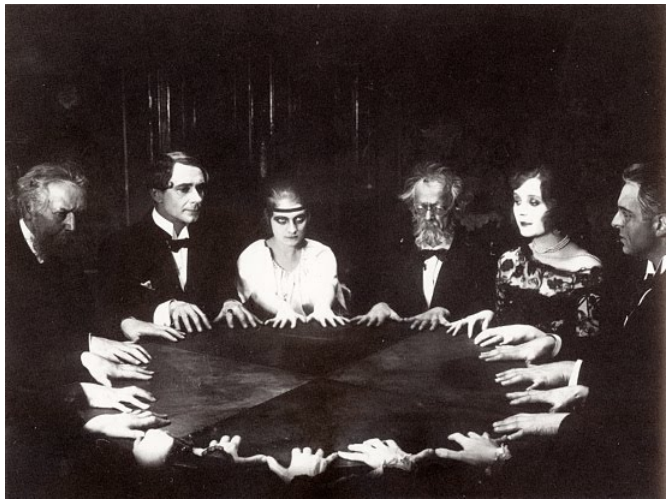
Upcoming cross validations in the next 3-6 months

- 2 analyte combo assay; CRO => CRO
- Single analyte pre-clinical; GSK => CRO
- Single analyte (pg/mL LLOQ); CRO => CRO
- Single analyte; CRO => CRO
- Parent + 5 metabolites (!); CRO => CRO
- Single analyte GSK => CRO
- Single analyte GSK => CRO
- Parent + 2 metabolites pre-clinical; GSK => CRO

Stop and Reflect

…ask someone for directions

- Learn from recent experiences and modify process if needed
  - Appropriate (lack of) informed consent has caused problems => QC only cross validations
  - Ensure that any requirement for diluting test samples is not blinded to the comparator lab
  - Investigating failures takes time => can be reduced by shipping certain items (e.g. stocks & working solutions) up front
  - Acceptance criteria can be met although a directional bias may still be evident
  - More test samples (in singlet) with ⅔rds within 20% acceptance criteria may be preferable



Alternate Proposal for Cross Validation

Under consideration based upon 9 months experience

Study Samples Available (3 options):

1. Select approx. 20 individual samples with original & ISR results at originator lab => re-test at comparator lab (in singlet)
2. Select approx. 20 individual samples with original result at originator lab => re-test at comparator lab (in singlet)
3. Study sample pools (approx. 20 but min. 12) prepared by originator lab and tested at both labs (in singlet):

- % difference ≤ 20% (mean) for at least ⅔ rds of the pooled samples (≤ 30% for LBA)
  - For options 1 & 2 use the original originator result
- Strongly advise the shipping of stock & working solutions plus study level QCs from originator lab to comparator lab
  - **Only** use as part of any subsequent investigation

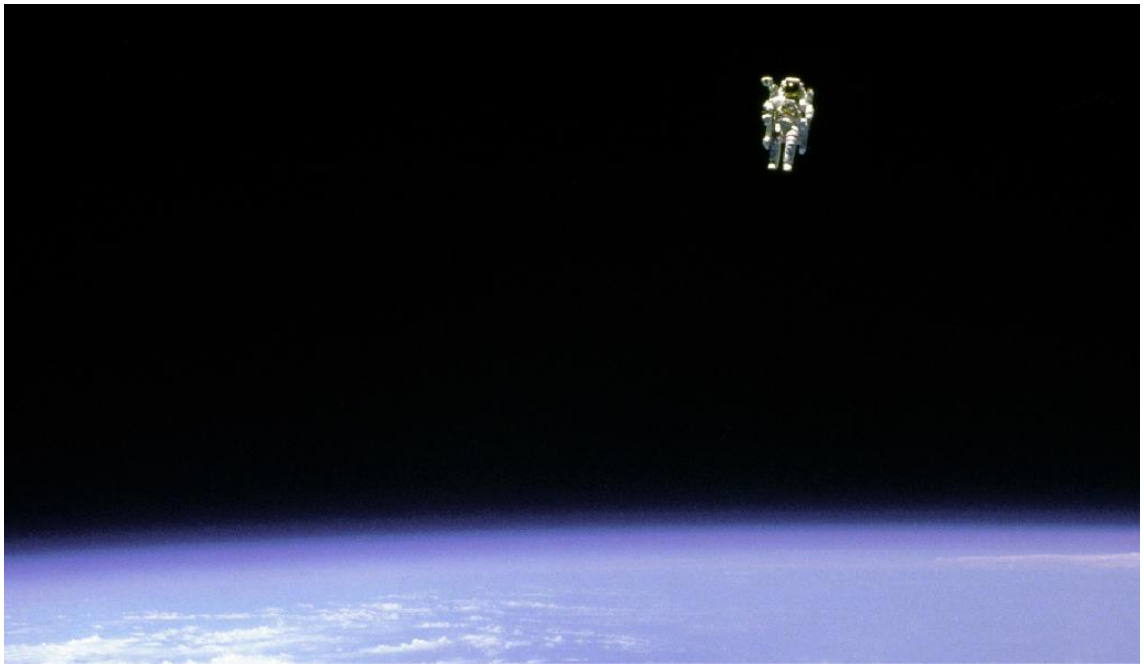
Alternate Proposal for Cross Validation

Under consideration based upon 9 months experience

Study Samples NOT Available:

- Study level QCs (i.e. low/mid/high) prepared by originator lab (ideally as part of previous study support)
  - Shown to be acceptable at the originator lab during study support (or a separate P&A run if prepared fresh for the cross validation)
  - Tested by comparator lab in reps of 6 (min. 3)
  - Usual QC acceptance criteria
  - CV ≤ 15% (≤ 20% for LBA)
  - Mean at each level within ±15% of nominal (±20% for LBA)

Are we nearly there yet?



- As strategy towards outsourcing bioanalysis increases (whole package), the need for sponsor => CRO cross validation may decrease
- CRO to CRO cross validation will probably still be required when running clinical studies in China
- Where the bioanalysis for “early” pre-clinical & clinical studies remains in-house, adoption of a tiered and pragmatic approach to assay validation (in-house) may be appropriate

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gsk
do more
feel better
live longer
Thank you