Workshop on ICH M10

Cross validation - working in the new paradigm

Tsvetelina Ivanova – on behalf of the EBF
(table moderator: Tsvetelina Ivanova, Matthew Barfield)

14 November 2023 – Barcelona, Spain

From the Guideline

- **2.2.3. Cross validation**
- Cross validation is required to demonstrate how the reported data are related when multiple bioanalytical methods and/or multiple bioanalytical laboratories are involved. (Refer to Section 6.2)

6.2. Cross validation

Cross validation is required to demonstrate how the reported data are related when multiple bioanalytical methods and/or multiple bioanalytical laboratories are involved.

Cross validation is required under the following situations:

- Data are obtained from different fully validated methods within a study.
- Data are obtained within a study from different laboratories with the same bioanalytical method.
- Data are obtained from different fully validated methods across studies that are going to be combined or compared to support special dosing regimens, or regulatory decisions regarding safety, efficacy and labelling.
- If data are obtained from different fully validated methods, and these data are not to be combined across studies, cross validation is not generally required.

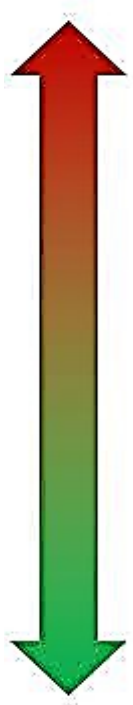
- Cross validation should be performed in advance of study samples being analysed, if possible.
- Cross validation should be assessed by measuring the same set of QCs (low, medium and high) at least in triplicate and study samples (if available) that span the study sample concentration range ($n \geq 30$) with both methods, or in both laboratories.
- Bias can be assessed by Bland-Altman plots or Deming regression. Other methods appropriate for assessing agreement between two methods (e.g., concordance correlation coefficient) may be used too. Alternatively, the concentration *vs.* time curves for study samples could be plotted for samples analysed by each method to assess bias.
- The use of multiple bioanalytical methods for the measurement of the same analyte in the conduct of one comparative BA/BE study is strongly discouraged.

Pre-meeting survey

	the question	Yes	No
Q1	Did you change your process of cross validation after ICH M10 became active?	16	18
Q2	Are you (still) applying acceptance criteria on cross validation?	16	10
Q3	If yes, which?		
Q4	If not, are you applying a correction factor for concentrations after cross validation?	1	10
Q5	If so, who does this? BA, PK scientist, other?		
Q6	If not, from when onwards do you consider two assays to be cross validated?		
free text	Do you also perform a partial validation exercise in the destination lab as well as a cross-validation?	8	2

Key message from the pre-meeting survey comments

- The new X-val process is not yet common practices in our industry (Q1)
- Many different interpretations on how to conduct and interpret the results from x-validation –
  - Many labs continue to use “ISR criteria” to interpret/accept X-val (as per Q3)
  - The practice of correction factor is not yet applied
- Additional guidance would help with regards to:
  - Results interpretation - **How?**
  - Results interpretation – **Who?**
- Good agreement that any correction factor resulting from statistical evaluations should not be applied by the BA team



Cross validation will also be discussed at the 16th OS

11:40 13:00 Session 20: Regulatory Updates - (Plenary) - Auditorium

11:40 12:30 ***Cross validation and ICH M10 - Case studies and Feedback from ICH M10 Workshop***

- 11:40 - 11:50: Case study 1: Richard Hughes, Resolian
- 11:50 - 12:00: Case study 2: Kamil Sklodowski, F. Hoffmann - La Roche
- 12:00 - 12:10: Case study 3: Daniël Splinter, argenx
- 12:10 - 12:30: Q&A and Feedback from ICH M10 Workshop

12:30 13:00 ***Focus on 3R - Feedback from ICH M10 Workshop***

- 12:30 - 12:40: Introduction to surrogate martix experiments for preclinicall assays
- 12:40 - 12:50: First results surrogate matrix experiments for preclinical chromatography assays
- 12:50 - 13:00: First results surrogate matrix experiments for preclinical Ligand Binding assays

Feedback from the round tables & discussions - 1

Comments:

- few companies are actually performing cross-validation
- majority are still applying ISR criteria mainly because:
  - It is easier
  - Lack of resources
  - Lack of stakeholder engagement
  - Sponsor request (CRO perspective)
  - Additional samples are needed for statistical evaluation

Actions: Sharing case studies about the x-validation conduct without an applying ISR acceptance criteria

Feedback from the round tables & discussions - 2

Recommendation:

- there is an inherent risk of more complex statistical procedures creeping into the bioanalytical toolbox; if/when Bland-Altman plots or Demming regression may not provide all the answers to interpret a cross
- We recommend to continue the discussion and share examples with industry and with regulators to ensure we all come on the same page and stay on the same page and do not overboard as a BioA community.

Raw data from the pre-meeting survey comments

- In the next slides we provide the unredacted details from 56 survey files reaching us prior to the deadline.
- Surveys that have arrived after the deadline could not be included anymore, for logistic reasons. Please speak up if your comment wasn't already captured in the other 56 files

On Q1: Did you change your process of cross validation after ICH M10 became active?

- not performed any cross validations x3
- Y, Removed acceptance criteria
- If incurred samples are not available for cross-validation is it recommended to prepare samples in unique lots of matrix or is a pool sufficient as long as encompassing dynamic range?
- N, No opportunity to carry out a cross val since M10 was introduced
- Y (study samples to be used in addition to QC samples)
- New crossval include overrange samples (both Incurred and spiked)
- To be implemented
- Took away the acceptance criteria
- Bland-Altman plot
- Y (30 studies samples)
- We would change it, not required yet
- Cross validation never implemented in our lab
- N (it was already in line with ICH M10)

On Q2: Are you (still) applying acceptance criteria on cross validation?

Yes	No
16	10

- not performed any cross validations
- moving away but difficult to convince people that things are different now; especially clinical pharmacology
- Depending on sponsor requirements
- to be synced with M10
- will be defined

Although majority has changed the approach with regards to x-validation, acceptance criteria are still widely used

On Q3: If yes, which?

- 66% within +/-20%
- ISR Criteria x9
- % difference less than 30% for 2/3 of total samples
- The CV% should be less than or equal to 15% for each QC level and laboratory/method/methodology . The mean accuracy at each concentration level should be between 85.0% and 115.0% of nominal concentration. The inter-laboratory trueness should be less than or equal to 20%.
- Qc and real study samples analysis acceptance criteria (% difference)
- % difference from the mean (20% for chrom, 30% for LBA)
- but we now look at bland altman plots as well as having ISR acceptance to look at trends. We test n=40 samples (30 incurred if available, 10 QCs and QCs have the same criteria as incurred samples - %difference rather than nominal) ISR criteria for study samples
- Chrom feedback: acceptance criteria +/-15% bias for blinded spiked QCed,
- within 15%
- Based on type of test samples(Nominal for Validation QC or % Bias for Study samples)
- Bias on QCs or incurred samples analysed at different labs
- Spiked QCs at 3 levels that overlap between methods applying acceptance criteria, plus a significant amount of incurred samples in the overlaping range (no acceptance for this one, although ISR % used as indication of performance between labs/ methods)

On Q4: If not, are you applying a correction factor for concentrations after cross validation?

- not performed any cross validations
- not done so far
- not sure, is for clinical pharmacology to decide; we are still in the phase of discussing and making them aware that things have changed with M10
- no examples of bias so far
- N - If correction factor is needed, this wouldn't be done by the CRO that performs bioanalysis.

- Correction factors are a NO NO, you are asking for trouble!
- if the statistical analysis showes a significant difference : Yes
- Case by case
- It depends on the PK model and the differences observed
- N, but Bland-Altman plot using percentage difference against concentration on a batch to batch basis or against PK timepoint, or by concentration plot. Correction factor might be consider by PK scientist

On Q5: If so, who does this? BA, PK scientist, other?

- Team decision
- it is not BA for sure
- Client (Statistician) to agree the correction factor
- Data manager/Sponsor
- PK scientist or Biometrics
- Clinical pharmacology
- clinical pharmacologist/ PK scientist in collaboration with BA

On Q6: If not, from when onwards do you consider two assays to be cross validated?

- on successful Q3
- when cross validation is completed
- As soon as the QCs and incurred samples have been run at the 2 labs the cross validation is "done" from a BA perspective
- Yes if no bias was seen.
- As soon as it is known that two assays or two laboratories are used for data creation.
- If assay validated pre M10 the method stands as it was OK at start of study.
- when acceptance criteria pass
- It needs to be assessed case by case, % difference can be a guidance but also how the difference can be explained in the PK model
- 2/3 of cross validation QCs within 15%
- I would like to know how evaluate in detail.
- As per M10 - if the study data are to be compared, a cross validation will be performed
- cross validation should be assessed, and incurred samples results should be compared and decision made on a case by case basis with Pkist support

Q – no number : Do you also perform a partial validation exercise in the destination lab as well as a cross-validation?

- Full Validation + Cross validation (as requested by M10 section 6.2)
- Yes, before performs a cross validation
- We are doing both although the partial maybe reduced and rely on the cross validation as additional evidence of assay validity.
- Decision is made by sponsor.
- Y (always full validation x3 + Assays are fully validated at the 2 sites
- run full validation in destination lab, then cross validate the assay
- yes, transfer and partial validation
- We have not been in that specific information, but I think it would be our way forward because you determine the P&A with the same QC's in both methods and evaluate the performance on greater concentration range by using clinical studies
- To date we have been the lab supplying the method/assay. Can’t answer for lab receiving the assay.
- If a method is developed in a lab but not validated then a transfer cannot be performed. Instead a method development is done at the destination lab. The method development can be based upon the already developed data from the original lab. "
- Y if two different labs within same organization are used. If different CROs full validation plus cross validation of the methods

And we have a few contributions

- Petya Milusheva, Comac Medical - Crossing the road of cross-validation
- Ali Maarouf, Sanofi - LBA Cross-Validation

Our interpretation to cross-validation – how?

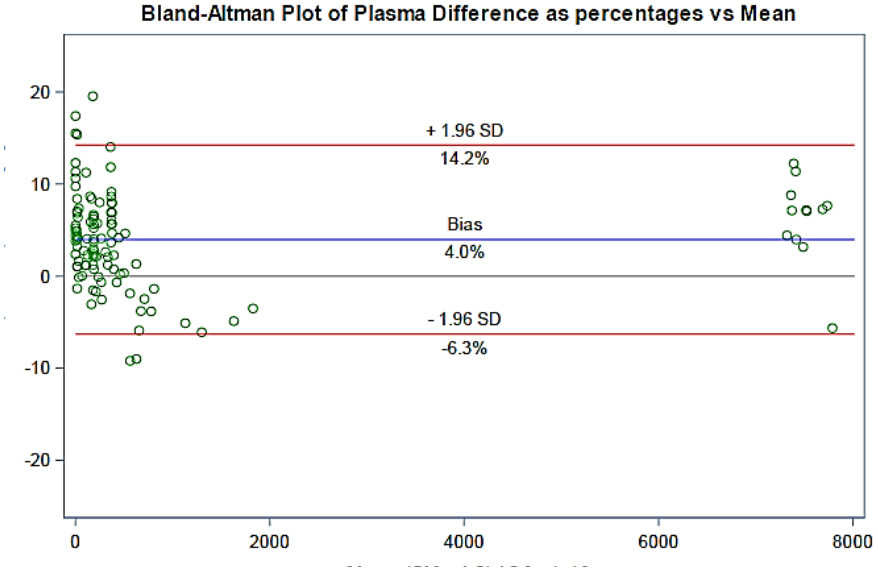
How to crossing the X-val road safely:

1. Number of samples to be analysed: determined using statistical approach based on the method(s) performance

2. Assessment method: Bland-Altman

3. ClinPharm involvement in:
   - Limits of agreement interpretation
   - Evaluation on impact on clinical data

Putting Theory into Practice…



- ❖ Sample Analyzed: 60 QCs and 51 study samples
- ❖ Bias: 4%
- ❖ Agreement Limits:

- Upper Limit: 14.2% (+1.96 SD)
- Lower Limit: -6.3% (-1.96 SD)

Our interpretation to cross-validation – challenges on the road?

1. Stakeholder engagement – why we need to cross the road together?

2. Overinterpretation – are we aligned on criteria when x-validation is expected?

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And we have a few contributions

- Petya Milusheva, Comac Medical - Crossing the road of cross-validation
- Ali Maarouf, Sanofi - LBA Cross-Validation

My question to the audience/to EBF is…

- In general, study samples are not available prior to sample analysis for the conduct of cross-validation as recommended by ICH M10. In lieu of analyzing study samples, is it accepted that n≥30 individual lots of matrix spiked with analyte would be suitable? What is the preferred method to demonstrate correlation? What would be an acceptable correlation for a successful cross-validation?

My process/our interpretation is

- Our current process for cross validation is to spike a single matrix pool with analyte at concentrations encompassing the dynamic range (LLOQ-ULOQ). Subsequently, study samples would be required to complete cross-validation. For comparison of cross-validation results, criteria from ISR is applied; 2/3 of samples must be within ±30% bias of each other for the cross-validation to be considered acceptable.

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