Workshop on ICH M10

L5 - Dilution QCs during sample analysis

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Introduction to Round table C6

4.2.6 Dilution Linearity and Hook Effect

" […] Dilution linearity should be demonstrated by generating a **dilution QC**, i.e., spiking the matrix with an analyte concentration above the ULOQ, analysed undiluted (for hook effect) and diluting this sample (to at least 3 different dilution factors) with blank matrix to a concentration within the calibration range. […]"

4.2.7 Stability

"[…] Since sample dilution may be required for many LBA methods due to a narrow calibration range, the concentrations of the study samples may be consistently higher than the ULOQ of the calibration curve. If this is the case, **the concentration of the QCs should be adjusted**, considering the applied sample dilution, to represent the actual sample concentration range. […]"

4.3.3 Calibration range

"At least 2 QC sample levels should fall within the range of concentrations measured in study samples. At the intended therapeutic dose(s)[…]"

Pre-meeting survey

	the question	Yes	No
Q1	Do you include dilution QCs as standard in sample analysis runs? (n=28)	6	22
Q2	if yes, for what reason? (legacy decision, trust, company policy, or interpretation, upon sponsor or CRO request etc...)	N/A	N/A
Q3	Are they solely to cover dilutions outside of the validated bracket of dilution factors?	7	7
Q4	How many do you include and are they in addition to LMH? (Y/N refers only to the second part of the question)	0	8
Q5	What are the acceptance criteria and If they fail, do you reject the whole plate of diluted samples?	N/A	N/A

Key message from the pre-meeting survey comments

- Do you include dilution QCs as standard in sample analysis runs?
  - Good agreement among the community regarding NOT using them
- if yes, for what reason? (legacy decision, trust, company policy, or interpretation, upon sponsor or CRO request etc...)
  - Samples always need to be diluted
  - DilQCs are like any other QCs
  - Process control
  - Interpretation of ICH chapter 4.3.3
- Are they solely to cover dilutions outside of the validated bracket of dilution factors?
  - Different interpretations among the community (50-50)
- How many do you include and are they in addition to LMH?
  - 1 to 3 sets, always in addition to LMH
- What are the acceptance criteria and If they fail, do you reject the whole plate of diluted samples?
  - Same as normal QCs.
  - Good agreement about not rejecting the all run but just the samples that have the corresponding dilution

Key message from the workshop

Key message from the workshop

- Do you include dilution QCs as standard in sample analysis runs?
  - >80%: Good agreement among the community regarding NOT using them
- If yes, for what reason? (legacy decision, trust, company policy, or interpretation, upon sponsor or CRO request etc...)
  - Samples always need to be diluted
  - DilQCs are like any other QCs
  - Process control of analyst errors during dilutions
  - **4.3.3** “At least 2 QC sample levels should fall within the range of concentrations measured in study samples.”
- Are they solely to cover dilutions outside of the validated bracket of dilution factors?
  - Different interpretations among the community (50-50)
- How many do you include and are they in addition to LMH?
  - 1 to 3 sets, always in addition to LMH
- What are the acceptance criteria and If they fail, do you reject the whole plate of diluted samples?
  - Same as normal QCs.
  - Mean value of 3 replicate QC within 20%CV/RE
  - Good agreement about not rejecting the all run but just the samples that have the corresponding dilution

The extra discussion and outlier comments

- Mis-interpretations: recommendation always to include dilutional QCs is only for chromatography.
- A positive change: Before M10 dil QCs was included, after M10 dil QCs is removed if samples included outside dilutional linearity range.
- An in study validation or partial validation: perform dilutional linearity, update method description SOP/validation report with the dilution factor and then use that dilution factor throughout.
- Small number of samples needing dilutions outside validation: included in study for each run.
- One company: Always include dilutional QCs and at each dilution level.

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

Q1: Do you include dilution QCs as standard in sample analysis runs?

No not as standard - we would use them if there is a need to dilute a sample by greater than the maximum validated dilution factor.	No, not as standard.	Y (pre-clinics)	N, not if validated

Q2: if yes, for what reason? (legacy decision, trust, company policy, or interpretation, upon sponsor or CRO request etc...)

samples always need to be diluted	DiQC are like any other QC, if you remove them why nt remove all QCs?	Dilutional plus procedure control involved.	company policies to control dilution applied during sample analysis	process control

SOPs vendor	Some clinical samples has concentrations above the ULQQ.	according to 4,3,3 interpretaiton	To support the dilution steps, i aim to have one set per dilution or group

Q3: Are they solely to cover dilutions outside of the validated bracket of dilution factors?

NA, we do not have had the need to analyse outside the validated range/bracket	In case not validated	N - Dilution was validated in dilution linearity Usually dilution QCS are added to mimic actual dilutions that will be applied to samples - analysis samples grouped per dilution	If a dilution factors outside the validated dilution factors are used, then a dilution QC is prepared to cover the accuracy and precision of this dilution factor.	Most commonly

Q4: How many do you include and are they in addition to LMH?

would need to check	3 replicates (within-run assessment criteria) to validate the dilution factor (outside the validated range), in addition to LHM	yes an additional set (front and back) of high concentration samples diluted independently to siome point between the Low-High range of concentration	2 replicates in addition per level	2 samples at 1 conc level
minimum one, in addition to LMH	3 in addition	1 diltuion QC in addition to Low-Medium high - in practice this has never been needed	2 in addition	1 set in duplicate

Q5: What are the acceptance criteria and If they fail, do you reject the whole plate of diluted samples?

20%/25%	I believe there are no extra dilution QCs included; it's just that plate acceptance QCs are at a conc which after dilution fall at the specified levels within the calibration range (low, mid, high)	Only diluted samples	both diluted QCs would need to pass criteria, +/- 20% etc. if one fails then only samples at the additional dilution would be rejected.	Regular, similar to LMH. Y, reject diluted samples in case corresponding DIL QCs are rejected.
Same as QC, as the quality/standard of reported data msut be the same throughout.	Diluted samples rejected	20% and yes, reject these DF samples of this run	1 out of 2 can fail. If both fail, reject diluted study samples	use as criteria to reject diluted samples if fails
2 out of 3 must pass / no rejection criteria on plate on samples, discussion with sponsor when failing	I reject only the failed diluted samples.	plate accepted, diluted samples rejected	Reject the diluted samples	we only reject samples with that dilution

Q5: free comments

Dilution linearity is assessed during assay validation. If sample dilution factors are covered by the highest dilution assessed during validation, nothing else is done.	At some vendors: Dilution Quality Control (DQC) samples are used for acceptance of diluted study samples at the respective dilution level. The dilution level is acceptable if at least two-thirds of the DQC samples, when diluted in to range, are within ±20.0% of their nominal concentration.