Workshop on ICH M10

C4 - Dilution QCs during validation & sample analysis

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14 November 2023 – Barcelona, Spain

From the guideline

3.2. Validation

3.2.7. Dilution integrity

- Dilution integrity is the assessment of the sample dilution procedure, when required, to confirm that it does not impact the accuracy and precision of the measured concentration of the analyte. The same matrix from the same species used for preparation of the QCs should be used for dilution.
- Dilution QCs should be prepared with analyte concentrations in matrix that are greater than the ULOQ and then diluted with blank matrix. At least 5 replicates per dilution factor should be tested in one run to determine if concentrations are accurately and precisely measured within the calibration range. The dilution factor(s) and concentrations applied during study sample analysis should be within the range of the dilution factors and concentrations evaluated during validation. The mean accuracy of the dilution QCs should be within ±15% of the nominal concentration and the precision (%CV) should not exceed 15%.
- In the cases of rare matrices, use of a surrogate matrix for dilution may be acceptable. It should be demonstrated that this does not affect precision and accuracy.

From the guideline

3.3. Study sample analysis

3.3.2. Acceptance criteria for an analytical run

- Analytical runs containing samples that are diluted and reanalysed should include dilution QCs to verify the accuracy and precision of the dilution method during study sample analysis. The concentration of the dilution QCs should exceed that of the study samples being diluted (or of the ULOQ) and they should be diluted using the same dilution factor. If multiple dilution factors are used in one analytical run, then dilution QCs need only be diluted by the highest and lowest dilution factors. The within-run acceptance criteria of the dilution QC(s) will only affect the acceptance of the diluted study samples and not the outcome of the analytical run.

Introduction to Round table C4

When?

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Introduction to Round table C4

How?

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Pre-meeting survey

	the question	Yes	No
Q1	Do you include dilution QCs as standard in sample analysis runs?	29	8
Q2	if yes, for what reason? (legacy decision, trust, company policy, or interpretation etc...)		
Q3	Are they solely to cover dilutions outside of the validated bracket of dilution factors?	7	22
Q4	How many do you include and are they in addition to Low-Medium-High?		
free text			

Key message from the pre-meeting survey comments

- Do you include dilution QCs as standard in sample analysis runs?
  - 78% include dilution QCs today when diluted samples are included
- if yes, for what reason? (legacy decision, trust, company policy, or interpretation etc...)
  - legacy decision, company policy
  - ICH M10 requirements
  - quality check of sample dilution, good practice
- Are they solely to cover dilutions outside of the validated bracket of dilution factors?
  - 76% No, dilution QCs are always included when samples are diluted
    - handled as process controls for each sample dilution within a run
    - dilution factor used for sample analysis in that particular run/batch
    - handled as regular QCs
- How many do you include and are they in addition to Low-Medium-High?
  - in addition to Low-Medium-High
  - 75% of responses apply 2 replicates, remaining triplicates (2/3 passing) and one up to 4 replicates
  - Majority of companies include a Dilution QC level per DF, only few apply the bracketing approach (lowest and highest dilution)

Key message from the pre-meeting survey comments

- Free text in the survey responses show that there a still quite some questions related the HOW

  - Why validating a dilution factor during validation, when it is validated within each run?
  - Separate dilution QCs are prepared, is it appropriate to use the QCH and add a dilution factor?
  - Which level is your preferred level for the dilution QC - exceed study sample level or above the ULOQ level? Is 2xULOQ sufficient
  - What concentration of a dilution QCs used when multiple dilutions are applied within a run?
  - How to conclude on results if we have low level diluted QC and high level diluted QC (bracketing approach), and one of the two are not validated ?
  - When to perform dilution QC stability, up front in validation, or only when observed in sample analysis?
  - Is there any feedback from HA related dilution QC approach?

Let's discuss the HOW

- What is the recommended concentration for DiQC(s)?
  - exceed study sample concentration level or above the ULOQ level
  - How do you handle stability assessment of these DiQCs?

- Is a bracketing approach applying the lowest and highest dilution within a run/batch recommended?
  - How to handle dilutions when one of these DiQC levels fail?
  - If so, the process control element is lost, isn't it?

- When during sample analysis it becomes obvious that the original DFs are not sufficient, how is a new level implemented, using 5 replicates?
  - Will additional stability required for a higher DiQC concentration?

- Any feedback from Health Authorities related this topic?

Feedback from the round tables

Theme/question: Recommended approach and conc for dilQC?

Comments:

- Most doing dilQCs in sample analysis – most duplicate, some triplicate (2/3 rule)
- Validation per Dil Factor in the guidelines?
  - really needed? → scientifically, a lower Dil Factor could be covered by a higher Dil Factor – Action?
  - Most try to minimise number of different Dil Factors validated – Recommendation
- What concentration dilQC is prepared?
  - mixture of responses, 20 fold, 10 fold, 2-5 fold, depends on required Dil Factor and type of study
  - min dilution (but has to be above range) – 1.5 fold generally used (to cover limited sample volume)
- DilQC Stability?
  - nothing in M10 explicitly stating DilQC stability, so only needed to cover DilQC storage
  - However also used practically to cover sample stability

Theme/question: Further dilution during sample analysis – further validation?

Comments:

- If needing additional Dil Factor (not validated) – where is it performed?
  - Mostly in sample analysis (n>5)
  - Some in validation – cross reference for different studies using the method and a cleaner submission of studies that are required
- Running of Dil Factor in sample Analysis
  - Limited benefit outside of process control
  - any benefit of Bracketing approach? Process for accepting other not-tested Dil Factors when one of the tested Dil Factors fails?
- Limited HA feedback (1 case)
  - Validated 1 in 4 DF, but ran 1 in 2 Dil Factor in sample analysis, was asked to go back and validate 1 in 2 Dil Factor

Recommendation: don't make your life too complex,
Are DilQCs really required?
And is there really a need to validate each Dil Factor ?

Raw data from the pre-meeting survey comments

- In the next slides we provide the unredacted details from 37 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 37 files

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

Yes	No
29	8

- Only for diluted sample runs
- Only in case study samples are diluted
- No, Only when dilutes samples measured
- Y, Not carried out if there are no sample dilutions in the sample run.
- only when samples are diluted in the run
- Yes, if dilution of study samples are planned
- was SOP specific for CROs until now
- only when dilutions are present in the run
- Not yet, but it will be done soon
- Yes when needed
- Y - Only when unknown samples are diluted in the same run
- Y (Runs involving samples with diution)
- If sample is to be diluted, then we would add the dilution QC. if no dilutions required for the samples, then no dilution QC added.
- not before ICH M10 if not part of the vendor's policy, now yes if there are diluted samples

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

- only if samples diluted
- interpretation of ICH M10 (page 20)
- in process control
- It's explicit in 3.3.2
- all
- Accordingly ICH M10
- required as part of the guidance
- legacy decision
- according to ICH M10
- Only included in runs with diluted samples to demonstrate adequate sample dilution
- Legacy and "quality", the dilution procedure needs checking
- I see DIQC the same as regular QC. All or nothing!

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

- company policy
- if samples required dilution
- it's mentioned in the ICH M10
- As QCs
- good practice, interpretation
- the mutual interpretation of many participants at the workshops/meetings in Barcelona 2022
- legacy decision
- Only if diluted samples are analyzed
- Runs involving samples with dilution
- good practice, interpretation
- As additional control for df applied to unknows samples out of calibration range
- As per M10 - but also to implement an additional check that the pipetting in preparing that dilution factor was not off.
- because of the ICH M10,

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

- No, They are QCs
- N, They are process controls required only if you're carrying out sample dilutions.
- no, to cover the actual dilutions applied to study samples in a run any dilution as it is acting as a process control
- was SOP specific for CROs until now N, we understand that the process and possible errors during dilution should be checked, not only in case of ULOQ results
- They are also added as run control for data outside the curve
- No it is the dilution factor used for sample analysis in that particular run/batch
- not clear the question, if there are diluted samples, Dil QC should be present for acceptance criteria

On Q4: How many do you include and are they in addition to Low-Medium-High?

- in Duplicate, additional to L, M, H
- Addition to L-M-H and DQC (n=2) per dilution factor
- Same number of replicates as for Low-Medium-High
- Depend on dilutions used
- triplicate, 2/3 must pass, in addition
- Additional to L, M, H if Dils are required
- A QC with two times QC-HIGC conc is used as dilution QC.
- 2 QC's per dilution factor.
- In addition to L-M-H Qc levels (2 replicates)
- for all dilution factors in a batch as well as L-M-H
- 2 QCs per dilution (bracketing lowest and highest if required); in addition to QC low-medium-high
- 2 reps, 1 additional level expected conc. of the analyte
- in addition to the L-M-H, 4 replicates are included for each dilution

On Q4: How many do you include and are they in addition to Low-Medium-High?

- 2 per run
- 2 xDiQC
- One needs to be OK.
- 3 replicates in addition per level
- Depends on how many factors are needed, yes in addition to Low, Med, High "2 replicates by dilution factor
- in addition to L-M-H QC's"
- 2 DILQC at one concentration level in addition
- number depends on the dil factors applied - yes in addition of L,M,H
- DQC for each dilution factor in 3 replicates
- Minimum of two samples per dilution factor analysed. However we now use as few different dilution factors per run as possible
- 1 factor in triplicate
- Included only when applying dilution for subject sample
- Additional and in equal number of Low-medium and high

On Q4: How many do you include and are they in addition to Low-Medium-High?

- Yes, it will depend on the dilution factors required for the run. Might be adding the additional 1:1 dilutions as well as the 1:10 dilutions required. Yes this is as an additional QC to the HIgh, Med, Low QC.
- SOP
- 1 dilution QC in addition to Low-Medium high - in practice this has never been needed
- DQc for each dilution factor in 3 replicates
- We include dilution QCs (in duplicate) that need a different dilution factor to be assessed in addition to L, M and H QCs
- Add only the dilution factor used for sample analysis. And they are in addition to Low-Medium-High
- from 2 to 3 depending on amount of samples and vendor's SOP

Free text

- Used as standard if there are samples to be diluted.
- Further question request - Are you performing dilution QC stability up front in the validation, or only when observed in sample analysis?
- When using multiple dilutions (>2) then a bracketing approach is used, i.e. 4 replicates for lowest dilution and 4 replicates for highest dilution.
- Using one level above the ULOQ (one sample) for both dilution.
- This part is unclear : 1) in validation, we have to validate one dilution factor (the highest we assumed) 2) in assays, we have to validate each dilution factor used during each analytical run. What is the purpose of doing it in validation, so ? And how to conclude on results if we have low level diluted QC and high level diluted QC, and one of the two are not validated ?
- Currently separate QCs (OR) is prepared for the dilution QC. Is is appropriate to use the QCH and add a dilution factor? Or does it absolutely have to be a separate QC specially prepared for dilutions?