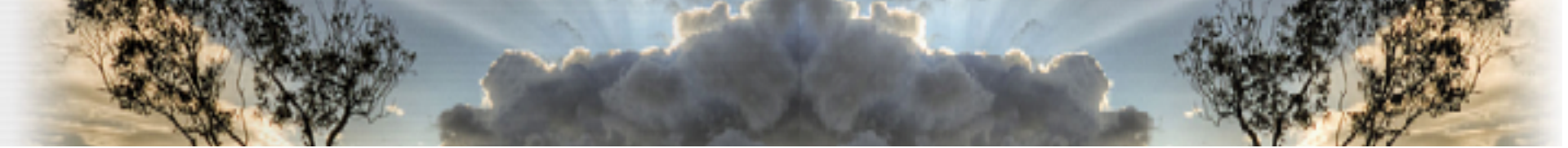

Stability Assessment

Michaela Golob, on behalf of the EBF

ICHM10:
EWG-Summary of Guideline Content (LBA): Stability

Stability should cover the processing and storage conditions of the study samples.

- At least 3 low and 3 high concentration QCs should be analysed at time zero and after the applied storage condition.
- The mean accuracy should be within ±20% of the nominal concentration at each QC level.
- Stability tests should be evaluated for freeze-thawing the matrix, bench top stability and long term stability.
- It is acceptable to apply a bracketing approach, e.g., demonstrated stability at -70ºC and at -20ºC, also applies to in between temperatures

Comparable to current guidelines (EMA, MHLW, FDA)

- Bench-top stability (adapted to assay needs)
- Freeze/Thaw stability (adapted to study sample needs)
- Long Term Stability (adapted to study sample needs)
  - Min 3 replicates of low and high QC level
  - Acceptance criteria:

    mean concentration at each level within ±20% of nominal

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Changes to current guidelines (EMA, MHLW, FDA)

- Multiple stability QC preparations (based on HC)
- Use of fresh QCs (based on FDA)
- High stability QC concentration adapted to C_{max}
- -20ºC stability validates also lower temperatures for chemicals, bracketing approach -20ºC to -70ºC for biologics
- [Whole blood stability]

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Multiple stability QC preparations Paragraph from ICH M10

Stability of the analyte in the studied matrix is evaluated using low and high concentration stability QCs. Aliquots of the low and high stability QCs are analysed at time zero and after the applied storage conditions that are to be evaluated. **A minimum of three stability QCs should be prepared and analysed per concentration level/storage condition/timepoint.**

Changes to current (EMA, MHLW, FDA)

- ICHM10 text is based on HC requirement
- Different to other current local guidelines of EMA, MHLW and FDA as well as industry proposal for ICHM10 in 2016

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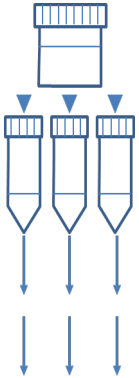
Changes to current (EMA, MHLW, FDA)

Tube numbers in QC preparation of stability testing:

ICHM10



Blank matrix + analyte

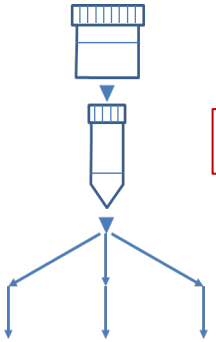
3 aliquots

Storage

Analysis

3 measured values

EMA, MHLW and FDA as well as industry proposal for ICHM10 in 2016



Blank matrix + analyte

1 aliquot

Storage

Analysis

3 measured values

EBF discussion & perspective

- Definition on what is requested in practice by "…three stability QCs are prepared…"
- Independent preparations of stability QCs per concentration level/storage condition/timepoint will require
  - more matrix, is this ethical (3R!)
  - more preparation time and storage space

Multiple QC preparations in stability tests will increase validation effort, but will not increase data quality.

EBF perspective:

QC preparation should be performed with one aliquot for storage, analysed 3 times.

Use of fresh QCs
Paragraph from ICH M10

…The stability QCs are analysed against a calibration curve, obtained from freshly spiked calibration standards in a run with its corresponding freshly prepared QCs or QCs for which stability has been proven. While the use of **freshly prepared calibration standards and QCs** is the preferred approach, it is recognised that in some cases, for macromolecules, it may be necessary to freeze them overnight. In such cases, valid justification should be provided and freeze-thaw stability demonstrated. The mean concentration at each level should be within ±20% of the nominal concentration…

Changes to current (EMA, MHLW, FDA)

EMA Section 4.1.9:

“Stability of the analyte in the studied matrix is evaluated using low and high QC samples (blank matrix spiked with analyte at a concentration of a maximum of 3 times the LLOQ and close to the ULOQ) which are **analysed immediately after preparation and after the applied storage conditions that are to be evaluated**. The QC samples are analysed against a calibration curve, obtained from freshly spiked calibration standards, and the obtained concentrations are compared to the nominal concentrations. The mean concentration at each level should be within ±15% of the nominal concentration.

section 7.1.1.11:
The mean concentration at each level should be within 20% of the nominal concentration.

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Changes to current (EMA, MHLW, FDA)

FDA:

"Matrix-related stability experiments should compare stability QCs against freshly prepared calibration curves and freshly prepared QCs. Although the use of freshly prepared calibrators and QCs is the preferred approach, in some cases, (e.g., for macromolecules), it may be necessary to freeze them overnight. In such cases, the sponsor should provide valid justification and demonstrate the freeze-thaw stability."

!Glossary:
Freshly prepared: Freshly prepared refers to QC sample preparation (i.e., spiked) on the day of the experiment; not frozen before use.

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EBF discussion & perspective

- What is meant by “freshly prepared QCs”?
- QC samples do represent the study samples therefore they need to be treated the same as study samples. => Mostly all our study samples in regulated bioanalysis are frozen, so run acceptance QCs cannot be “**fresh**” in the sense of “never frozen” as stated in the FDA guideline glossary.
  => "Valid justification" if QCs are frozen - they mimic the unknowns

- QC samples for stability assessment cannot be **long time stored frozen** (explicitly not longer than stability QCs)

EBF perspective:

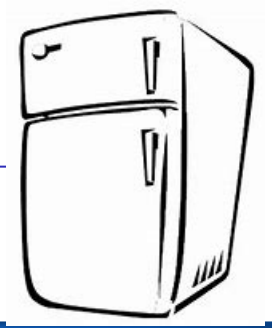
Clarify what is meant by “freshly prepared QCs”

QCs should represent the samples and these are never (99%) fresh, QCs should therefore always be freshly prepared but at least frozen overnight so there is no interference with stability assessment.

Stability at one storage temperature Paragraph from ICH M10

…For chemical drugs, it is considered acceptable to extrapolate the stability at one temperature (e.g., -20° C) to lower temperatures (e.g., -70° C).

For biological drugs, it is acceptable to apply a bracketing approach, e.g., in the case that the stability has been demonstrated at -70° C and at -20° C, then it is not necessary to investigate the stability at temperatures in between those two points at which study samples will be stored...



Stability at one storage temperature

Changes to current (EMA, MHLW, FDA)

- EMA

For small molecules it is considered acceptable to apply a bracketing approach, i.e. in case stability has been proved for instance at -70ºC and -20ºC, it is not necessary to investigate the stability at temperatures in between.

For large molecules (such as peptides and proteins) stability should be studied at each temperature at which study samples will be stored.

- MHLW:

The stability of the analyte should be assessed under conditions that are as close as possible to the actual circumstances, e.g. sample storage and sample analysis.

- FDA:

The storage temperatures studied should be the same as those used to store study samples.

EBF discussion & perspective

- Demonstration of frozen storage stability at one temperature and time −20ºC validates the use of lower temperatures for storage for the same period of time.
  Scientifically this is applicable for both chemicals as well as biologics – variable values seen might not be a real storage issue but assay variation
- Any evidence that extrapolation is not applicable for biological drugs?
  - All data collected by EBF members support extrapolation of the stability at one temperature (e.g., -20ºC) to lower temperatures (e.g., -70°C). (Data shown Susanne Phil)
  - Apparently regulators have cases where stability is different at lower temperatures. Is this just assay variability? Is this the exception? ICHM10 should not focus on the exception rather than norm.

EBF perspective:

Stick to the FDA recommendation: "determination of stability at -20 would cover stability at colder temperatures".

High concentrated stability QCs
Paragraph from ICH M10

…Since sample dilution may be required for many LBA assays 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 stability QCs should be adjusted, considering the applied sample dilution, to represent the actual sample concentration range…

High concentrated stability QCs

Changes to current (EMA, MHLW, FDA)

- Not covered in other guidelines

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EBF discussion & perspective

- In case of pivotal tox study support the highest sample volume is often not known at the time of validation: Could this only apply to BA/BE studies?
- Should stability testing of samples >ULOQ be repeated at a later time-point in the assay life cycle?
- Is this than adding an additional upper high QC mimicking the actual sample concentration of the study samples in addition to the low and high stability QC?
- Normally low concentrations are stability indicating. It is very unlikely for a high concentrated sample failing stability if low concentration sample is stable
- High concentration sample testing is are already included in dilution linearity and Hook effect assessment
  - Blank matrix sample spiked at or above anticipated Cmax or at highest feasible concentration
  - At least one spike >ULOQ (for hook) and 3-5 dilutions within assay range

EBF perspective:

No need for adjusted high stability concentration QC

Whole blood stability for LBA
Paragraph from ICH M10

"Stability evaluations should be carried out to ensure that every step taken during sample preparation, processing and analysis as well as the storage conditions used do not affect the concentration of the analyte."

(CC part 458-465: Whole blood stability)

EBF discussion & perspective

- Section 787-789: Is whole blood stability expected for LBA?
  - LBA: Stability assessment cannot be performed by direct analysis of whole blood as there is no assay for blood
  - No need to perform method in blood if your matrix is serum
  - GBC publication:

Nico van de Merbel et al: “Stability: Recommendation for Best Practices and Harmonization from the Global Bioanalysis Consortium Harmonization Team” (2014)

“Stability assessment is generally not necessary if stability in plasma/serum has been demonstrated under the same conditions unless the analyte is known to behave differently in the presence of blood cells”

EBF perspective:

It should be stated in the guideline that whole blood stability is not required for LBA.

Suggested comment to EMA/EWG

Final recommendation from this presentation, which combines the original recommendation enhanced with the discussions from the panel discussions during the meeting, are captured in the summary slide deck: Recommendations from the EBF Spring FW 2019

Acknowledgement and questions

- EBF community and ICH M10 focused workshop delegates for their feedback

- Any questions: please send questions to info@e-b-f.eu, before 31 May for consideration in meeting feedback to EMA/EWG