Workshop on ICH M10

C2 - Whole blood stability for Chrom

Jörg Faber – on behalf of EBF
(table moderator: Jörg Faber & Enric Bertan)

14 November 2023 – Barcelona, Spain

Introduction to Round table C2

The scope of C2 = whole blood stability for Chrom

Before we start

3.2.8. Stability

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.

The storage and analytical conditions applied to the stability tests, such as the sample storage times and temperatures, sample matrix, anticoagulant and container materials, should reflect those used for the study samples. Reference to data published in the literature is not considered sufficient. Validation of storage periods should be performed on QCs that have been stored for a time that is equal to or longer than the study sample storage periods. Stability of the analyte in the matrix is evaluated using low and high concentration QCs. Aliquots of the low and high QCs are analysed at time zero and after the applied storage conditions that are to be evaluated. One bulk QC should be prepared at each concentration level. For each concentration tested, the bulk sample should be divided into a minimum of 3 aliquots that will be stored, stressed and analysed. …

Introduction to Round table C2

Before we start

3.2.8. Stability

… The QCs should be analysed against a calibration curve, obtained from freshly spiked calibration standards in a run with its corresponding freshly spiked QCs or QCs for which stability has been proven. The mean concentration at each QC level should be within ±15% of the nominal concentration. If the concentrations of the study samples are consistently higher than the ULOQ of the calibration range, the concentration of the high QC should be adjusted to reflect these higher concentrations. It is recognised that this may not be possible in nonclinical studies due to solubility limitations.

[…]

Introduction to Round table C2

Before we start

3.2.8. Stability

[…] In addition, the following test should be performed if applicable:

4) Stability of the Analyte in Whole Blood

Sufficient attention should be paid to the stability of the analyte in the sampled matrix (blood) directly after collection from subjects and prior to preparation for storage to ensure that the concentrations obtained by the analytical method reflect the concentrations of the analyte in the subject's blood at the time of sample collection.
If the matrix used is plasma, the stability of the analyte in blood should be evaluated during method development (e.g., using an exploratory method in blood) or during method validation. The results should be provided in the Validation Report.

Introduction to Round table C2

When?

3.2.8. Stability

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Introduction to Round table C2

How?

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Pre-meeting survey

	the question
Q1	Do you feel the paragraph on when blood stability is required is clear?
Q2	if no, what are the ambiguities you see?
Q3	Do you feel the paragraph on how blood stability needs to be assessed is clear?
Q4	if no, what are the ambiguities you see?
free text	

Pre-meeting survey

	the question	Yes	No
Q1	Do you feel the paragraph on when blood stability is required is clear?	26	10
Q2	if no, what are the ambiguities you see?		
Q3	Do you feel the paragraph on how blood stability needs to be assessed is clear?	16	18
Q4	if no, what are the ambiguities you see?		
free text			

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free text			

- When is clear for 78%
- How is clear for 47%

Feedback from the round tables

Round table: C02 – Whole blood stability
Moderators: Jörg Faber / Enric Bertran

Question	Yes
ICH M10 compliance = Whole blood stability for BioA in human plasma, only?	~60%
Do we agree to ICH M10?	No
ICH M10 = general procedures of 3.8.2 also for WB stability?	Not clear!
Do we agree to ICH M10?	No
Do you know the EBF white paper on blood stability testing [1]?	~20%

[1]: Freisleben et. al, Bioanalysis (2011) 3(12), 1333-1336

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When to perform whole blood stability?

Comments:

- Also perform it in animal WB? ~50/50 split
- animals only, if there is doubt or previous knowledge
- Serum is questionable, but it's not the most common matrix for CHROM assays
- Use of whole blood without anticoagulant favoured over "surrogate blood" with anticoagulant
- Not everybody performed WB stability before ICH M10, some did/do it always

White paper recommendations:

One species (only extend if instabilities are observed)

For plasma and serum assays

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At what timepoint of drug development should it be investigated? (if we perform it only in human whole blood):

Comments:

- early as possible
- independent on of you just do human whole blood
- but most do it in validation

White paper recommendations:

During method development in prep of validation for the first GLP study

How to perform whole blood stability?

Comments:

- Plasma assay is used in most cases, because it's validated
- relative quantification *vs* T-zero
- 85-115% of T-zero is accepted
- QC_{low} and QC_{high}
- adjustment of QC_{high} is unclear

- Age and preconditioning is not expected to be in ICH M10
- WB should not be older than 5-7 days
- prewarming is done by all
- equilibration time before T-zero is diverse (10 min – 30 min)

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How to perform whole blood stability?

White paper recommendations [1]:

Blood should not be older then 24 h -> update?
Use whole blood instead of plasma -> this is done different, new data collection?
Prewarm blood to 37 ˚C before spiking -> confirmed
Reduce amount of organic solvent to a minimum when spiking -> not discussed
Use a qualified blood assay for absolute quantitation -> not favoured (see above)
Use same levels and acceptance criteria as for plasma -> confirmed

[1]: *Freisleben et. al, Bioanalysis (2011) 3(12), 1333-1336*

Key message from the pre-meeting survey comments

When:

- “if applicable” / How do you justify it is applicable or not?
- “Subjects”, are animals subjects?
- Animal fresh WB is hard to obtain
- Basically it stated always / We do it anyways in all cases
- Use your own SOP to define what you will do

How:

- It only defines "which" sample, i.e. freshly drawn
- No ambiguities, it is open for different scientific approaches / Use your own SOP to define what you will do
- No acceptance criteria and no assessment procedure are clearly defined / lack of details
- We need a method in blood?
- If performed during method development how can the data be reported?

- No experimental conditions as spiking at 37° C in whole blood (age of whole blood?), equilibration time after spiking

Let's discuss

- ICH M10 compliance = whole blood stability for BioA in human plasma, only?
  - Do we agree?
  - If so, to which timepoint of drug development should it be investigated?

- ICH M10 = general procedures of 3.8.2 also for WB stability?
  - Do we agree?

- Is the current EBF recommendation paper up to date?

On Q1: Do you feel the paragraph on when blood stability is required is clear?

- Yes, Preference to do it in the development
- Use your own SOP to define what you will do
- Be consistent
- halfway yes.
- We do it anyways in all cases
- Yes. We need to mimic clinical procedures to ensure that we stabilize the analyte if needed.
- Yes - assume here is meant 'whole blood stability'.

On Q2: if no, what are the ambiguities you see?

- It is not clearly stated blood stability is not needed for a preclinical assay
- Use of the term “if applicable”
- Use of the word “subject” - clinical only?
- Acceptance criteria
- Basically it stated always
- we have interpreted it to mean only conduct for plasma assays but with the wording 'if applicable': it is not clear what it means. An example is serum for which WBS cannot be determined experimentally
- which species should be used for whole blood stability.
- Only clinical as "subjects" are mentioned, but no animals
- WB stb to be assessed during dev or val ? / for non clinic and clinic ?
- should it be assessed in animals? Fresh WB is hard to obtain
- Guideline says "subjects" - are ‘animals’ subjects? Perhaps another example of industry being to stringent?
- Do we need to test WBS in all species, rat, mouse, dog or can we extrapolate data from one species?
- should it be assessed in animals? Fresh WB is hard to obtain
- the 'if applicable' might question if for precl. species this is needed? How do you justify if it applicable or not?

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On Q3: Do you feel the paragraph on how blood stability needs to be assessed is clear?

- Y, No details given, so own test can be made.
- N - does not go into the detail of the experiment taking into account partitioning
- Use your own SOP to define what you will do
- Be consistent
- you need a method in blood?
- Options could be useful, although it pertains to the lab to determine a adequate procedure
- Is there a "how"? It only defines "which" sample, i.e. freshly drawn?
- Options could be useful, although it pertains to the lab to determine a adequate procedure
- Yes - Industry seems to still stick to spike at Mid QC and do not read M10 which is saying spike at Low and High QC level.

On Q4: if no, what are the ambiguities you see?

- Levels not specified
- None, it is good that it is open for different scientific approaches
- using an exploratory method in a validation package
- No guidance. (e.g. Distribution of analyte into blood cells, possibility to use human whole blood)
- No acceptance criteria and no assessment procedure are clearly defined
- performed in validation or method development using exploratory methods, But if performed during method development how can the data be reported?
- how does this must be evaluated at the end, with or without calibration curve, e.g., Area (ratio), concentrations, …?
- how many replicates must be used and which concentration level. No experimental conditions as spinking at 37° C in whole blood (age of whole blood?), equilibration time after spiking
- lack of details

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On Q4: if no, what are the ambiguities you see?

- The example suggests a whole blood method is developed and evaluated
- May be add a comment to explain that this stability could be assessed by comparison of signal between T0 and Tx (obtained after extraction)
- can the plasma method be used?
- it's not clear if it has to be done or not in validation. The 2nd paragraph is not clear.
- Missing: concentration levels and replicates to be investigated
- Experiment design should be discussed in detail (e.g sample preparation, acceptance criteria based on response ratio etc.)
- Should Whole blood Stability be assessed as self referential or to be included within a run acceptance run? with STD&QC?
- Does blood matrix stability have to be done when later on using serum or plasma?
- it's not clear if it has to be done or not in validation. The 2nd paragraph is not clear.
- not clarity enough between use of plasma method to check stability in validation or different blood method during MDev, which criteria should be applied then?

Free text

- in vitro vs. ex vivo
- acceptance criteria
- number of replicates needed
- conditions should mimic the real conditions applied in the studies