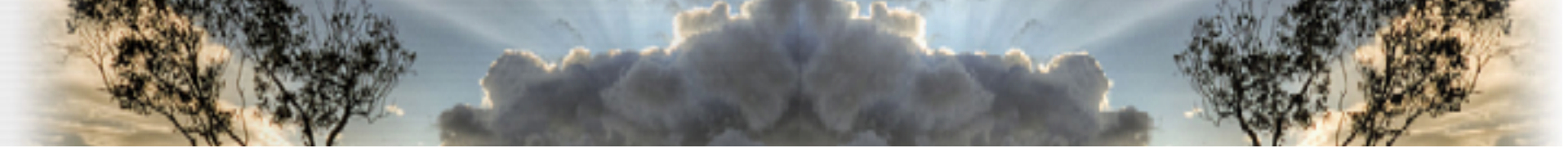

Haemolysed/hyperlipidaemic – matrix effects

Steve White, on behalf of the EBF

Paragraph from ICH M10

- A matrix effect is defined as an alteration of the analyte response due to interfering and often unidentified component(s) in the sample matrix. During method validation it is necessary to evaluate the matrix effect between different independent sources/lots.
- The matrix effect should be evaluated by analysing at least 3 replicates of low and high QCs, each prepared using matrix from at least 6 different sources/lots. The accuracy should be within ±15% of the nominal concentration and the precision (per cent coefficient of variation (%CV)) should not be greater than 15% in all individual matrix sources/lots. Use of fewer sources/lots may be acceptable in the case of rare matrices.
- The matrix effect should also be evaluated in relevant patient populations or special populations (e.g., hepatically impaired or renally impaired) when available. An additional evaluation of the matrix effect is recommended using haemolysed or lipaemic matrix samples during method validation on a case by case basis, especially when these conditions are expected to occur within the study.

Paragraph from ICH M10

- Haemolysed/hyperlipidaemic matrix also mentioned in Section 3.2.1 Selectivity
  - Tim Sangster will cover this in “Breakout session 2: Chromatography” after lunch
- However – useful to consider definitions used in Section 3.2.1:

“A naturally lipaemic matrix with abnormally high levels of triglycerides should be obtained from donors. Although it is recommended to use lipaemic matrix from donors, if this is difficult to obtain, it is acceptable to spike matrix with triglycerides even though it may not be representative of study samples. However, if the drug impacts lipid metabolism or if the intended patient population is hyperlipidaemic, the use of spiked samples is discouraged.

For the investigation of selectivity in haemolysed matrices at least one source of matrix should be used. Haemolysed matrices are obtained by spiking matrix with haemolysed whole blood (at least 2% V/V) to generate a visibly detectable haemolysed sample. ”

Changes to Current Guidelines

FDA	EMA	MHLW	ANVISA	China
In *Selectivity and Specificity* section	Matrix factor (MF) approach described by first intent	Not included	When the biological matrix is plasma, 8 (eight) samples of different sources must be analyzed, four of which are normal, two lipemic and two hemolyzed.	
No definitions of haemolysed or lipaemic matrix provided	If MF approach not possible (e.g. on-line sample prep) – then spiked QC (QC_{LOW} & QC_{HIGH}) approach		When the biological matrix is whole blood, six (6) samples from different sources, 4 (four) normal and two (two) lipemic, must be analyzed.	
No specific experimental design	No definitions of haemolysed or lipemic matrix provided		Matrix factor (MF) approach described	
			No definitions of haemolysed or lipemic matrix provided	
			Samples under study with degree of hemolysis superior to the degree of hemolysis approved in this test can not be analyzed.	

Impact on our industry – value for industry

- Matrix testing via QC approach rather than Matrix Factor is a welcome change – although 2 levels x 3 reps x 6 sources (excl. haemolysed & lipemic seems somewhat excessive)
- Definition of *'abnormally high levels of triglycerides'* is open to interpretation
- "Haemolysed matrices are obtained by spiking matrix with haemolysed whole blood (at least 2% V/V) to generate a visibly detectable haemolysed sample."
  - Implies that some kind of visual colour check of study samples may be expected
- Boundary testing approach may be appropriate, but no desire to test each and every study sample to ensure it falls within these boundaries
- For assays with SIL, this test could be viewed as redundant during validation as IS assessment during study runs should define sample acceptance
  - If scope is clear, then inclusion of these tests is more palatable
- "during method validation on a case by case basis, especially when these conditions are expected to occur within the study"…
  - Haemolysis could occur on any study
  - Lipaemic matrix may be easier to anticipate depending on study design
  - **Doing it for all validations will become "the norm"**

Previous EBF Position

- EBF White paper Bioanalysis (2014) 6(23), 3113-3120

  - Definition of hemolyzed matrix: ≥2% lysed blood in plasma
  - During validation: One source, QC Low, apply usual acceptance criteria.
  - If Pass: no need to inspect study samples.
  - If Fail: Consider further tests. Inspect study sample and the ones with hemolysis to be set to “NR”

  - Definition of hyperlipidemic matrix: Triglycerides > 300 mg/dL
  - During validation: One source, QC Low, apply usual acceptance criteria.
  - If Pass: no need to inspect study samples.
  - If Fail: Consider further tests. Inspect study sample and the ones considered hyperlipidemic to be set to “NR”

Frequency of Testing and Failure Rate

Chrom

	Preclinical	Clinical
Hemolysed tests	288	307
Failed tests	4 (1%)	7 (2%)
Hyperlipidemic tests	192	271
Failed tests	0	3 (1%)

LBA

	Preclinical	Clinical
Hemolysed tests	100	127
Failed tests	1 (1%)	3 (2%)
Hyperlipidemic tests	17	118
Failed tests	1 (6%)	2 (2%)

Source : EBF Focus Workshop in collaboration with the AAPS and JBF: industry input in ICH M10, Lisbon 2017

Feedback from EBF Strategy Meeting 2019

- Not clearly defined whether testing of haemolysed/hyperlipidaemic matrix is expected in matrix from single donors or whether pooled matrix is acceptable
- ‘abnormally high levels of triglycerides’ not defined and open to ambiguity
  - eg. triglyceride levels of > 300 mg/dL (3.4 mM/L)
- There should be an acknowledgement to the use of a SIL, which will compensate for any matrix effects from haemolysed/hyperlipidaemic matrix

Feedback from our delegates

- "*recommended on case by case basis*" is ambiguous
- It is acknowledged that triglyceride spiked matrix *“may not be representative of study samples*” – therefore why advocate this experiment at all? Either triglyceride spiked matrix is acceptable or it is not
- **Replace** *"Although it is recommended to use lipaemic matrix from donors, if this is difficult to obtain, it is acceptable to spike matrix with triglycerides even though it may not be representative of study samples".* **with** "*Certified naturally lipaemic matrix with levels of triglycerides above 300 mg/dL"*. Remove the possibility to use spiked samples for hyperlipidaemic matrix.
- Any expectation to test study samples fall within bounds of tests conducted is not viable
- Assess matrix impact in method development rather than validation

Proposals for Discussion

- Application for BA/BE studies only
  - For all other studies, a within study assessment of IS response is a scientifically sound approach
- Define ‘abnormally high levels of triglycerides’ to avoid any misunderstandings e.g. Triglyceride levels of > 300 mg/dL
- As it is acknowledged that spiking matrix with triglycerides may not be representative of study samples – so why do this test?
- Remove *“to generate a visibly detectable haemolysed sample”* from haemolysed matrix definition

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

Acknowledgment and Questions

- The EBF community for survey data and feedback
- Meeting delegates for survey data and feedback

- Please send questions to info@e-b-f.eu, before 31 May for consideration in meeting feedback