Hemolysed & Hyperlipidemic Plasma – a Real or Perceived Issue?

Presenter: Cecilia Sparr Eskilsson
on behalf of the EBF

Focus Workshop

(*In collaboration with the AAPS and JBF*)

Industry input into ICH M10: Experimental data as the cornerstone for a science driven bioanalytical guideline

The Altis Grand Hotel Lisbon,
Portugal September 24-26, 2017

Problem statement

- Recent guidance recommends investigations

Inconsistency in definitions of test matrices
Inconsistency in experimental design
Inconsistency in acceptance criteria & more…

Hemolysed and hyperlipidemic samples –
A real or perceived issue?

Hemolysed and Hyperlipidemic matrix tests

- BMV Guidances:
  - **FDA 2001**, no request to perform such tests
  - **EMA 2012**
    p. 8/22 section “Matrix Effects”:
    ”In addition to the normal matrix it is recommended to investigate matrix effects on other samples, e.g. haemolysed and hyperlipidaemic plasma samples.”
    p. 15/22 section “Selectivity”:
    ”Selectivity is tested by spiking at least 10 sources of sample matrix at or near the LLOQ. These sources should include lipemic and haemolysed samples.”

Hemolysed and Hyperlipidemic matrix tests

- EBF White paper Bioanalysis (2014) 6(23), 3113-3120
  (based on survey within EBF in 2013, literature research and discussions among BA peers)

  - 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”

Hemolysed and Hyperlipidemic matrix tests
Recent EBF surveys

- EBF Closed meeting in Nov 2016
  (Presentation by Birgitte Buscher based on surveys within EBF 2016)
  - 70/70% (SMOL/LBA) of members considered hemolyzed plasma tests in validations (30/20% only in clinical)
  - 70/50% (SMOL/LBA) of members considered hyperlipidemic plasma tests in validation, but only in clinical. (ca 20% for both preclinical and clinical)
  - Failure rates 1-20%.

- Survey within EBF in summer 2017
  - 27 companies responded (17 Pharmas, 10 CROs)
  - 50% uses definition of test matrices as EBF White paper.
  - Frequency of testing and failure rate (see next slide)

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%)

Data on study samples

- 75% respond that they do not statistics and do no checks.
- 25% share their “best guess”, most common guess <5%

Concerns

- There is still much differences in approaches (wrt. Definition of test matrices, definition of acceptance criteria, QC levels used, number of sources….)

- Survey data confirms self regulation in earlier stages of development by applying quite comprehensive experiments within validation

Recommended Best Practice Approach

- Based on data from several EBF surveys and the huge number of passed validation tests and low failure rates:

Assessment of hemolysed and hyperlipidemic matrix to be done in method development, not to be included in method validation.

If assessment during method development indicates impact on results, a partial validation can be conducted later when needed.

Acknowledgement

- EBF community
- Team members

  Marianne Scheel Fjording
  Jo Goodman
  Adrian Freeman
  Edwin Hooijschuur
  Brigitte Buscher
  Martina Wein
  Lorraine Jacobs
  Sorana Greiveldinger-Poenaru

Contact: info@europeanbioanalysisforum.eu