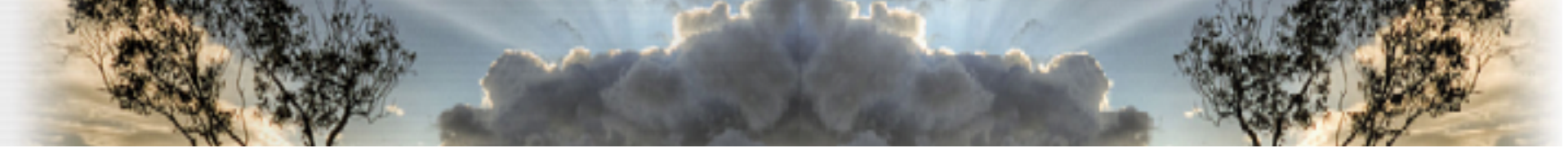


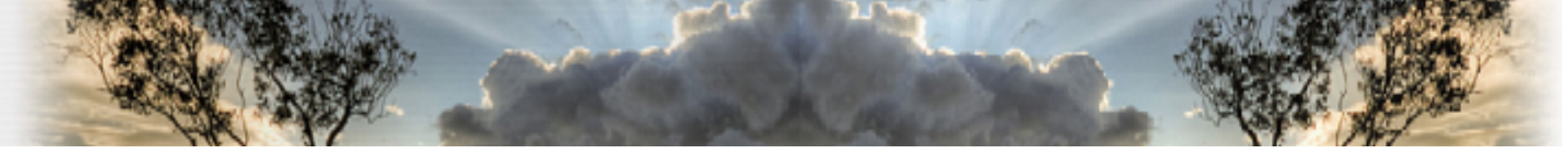
Specificity and Selectivity
4.2.1. + 4.2.2

Wibke Lembke, on behalf of the EBF

Specificity vs. Selectivity:

Specificity: Ability of an analytical method to detect and differentiate the analyte from other substances, including its related substances (e.g., substances that are structurally similar to the analyte, metabolites, isomers, impurities or concomitant medications).

Selectivity: Ability of an analytical method to differentiate and measure the analyte in the presence of interfering substances in the biological matrix (non-specific interference).



4.2.1 Specificity

4.2.1 Specificity

Specificity is evaluated by **spiking blank matrix samples with** related molecules **at the maximal concentration**(s) of the **structurally related molecule** anticipated in study samples.

The **accuracy** of the target analyte **at the LLOQ and at the ULOQ** should be investigated in the **presence of related molecules at the maximal concentration(s) anticipated in study samples**. The **response of blank samples spiked with related molecules should be below the LLOQ**. The **accuracy of the target analyte in presence of related molecules should be within ±25%** of the nominal values.

4.2.1 Specificity

In the event of **non-specificity**, the impact on the method should be evaluated by spiking increasing concentrations of interfering molecules in blank matrix and measuring the accuracy of the **target analyte at the LLOQ and ULOQ**. It is **essential to determine the minimum concentration of the related molecule where interference occurs**. Appropriate mitigation during sample analysis should be employed, e.g., it may be necessary to adjust the LLOQ/ULOQ accordingly or consider a new method.

During method development and early assay validation, these **"related molecules" are frequently not available**. **Additional evaluation** of specificity may be **conducted after the original validation is completed**.

Specificity: comparison to current guidelines

	ICH M10	EMA 2012	FDA 2018	MHLW 2014
Specificity	Interference of structurally related molecules anticipated in study samples	Interference by cross-reactivity with structurally related compounds or anticipated co-medications	Interference by cross-reacting molecules, co-medication, biotransformed species etc.	Cross-reactivity with coexisting related substances
Presence of related molecules to be added to	Blank and LLOQ + ULOQ in matrix	Blank and at LLOQ + ULOQ in increasing concentrations	calibration curves in buffer	Blank and near LLOQ + near ULOQ, to be tested at anticipated concentrations
Acceptance Criteria	Accuracy within ±25% for LLOQ and ULOQ Blank: BQL	Accuracy within ±25% for LLOQ and ULOQ	Accuracy within ±20% (±25% at LLOQ and ULOQ)	Accuracy within ±20% (or ±25% at LLOQ and ULOQ) Blank: BQL
Impact	Evaluated; minimum concentration where interference occurs needs to be determined; mitigation during sample analysis need to be employed		Should eliminate or minimize interference	Should be evaluated

Impact on our industry – value for industry

- To test at a high concentration control is scientifically justified as it allows formation of higher ordered complexes in the samples which might not be seen looking at LLOQ only

- In case of relevant co-medication specificity testing is useful, but not if structurally related molecule won`t be present in study samples

Feedback from EBF & Delegates

Include definition for specificity (harmonization between selectivity and chromatography)

Definition of “structurally related” molecules is missing and how to select and how many should be studied?

n= not defined

Harmonize ULOQ vs HQC for Selectivity

Wanted vs *unwanted* interference is not considered. *Wanted* interference becomes more important and is necessary for free analyte assays

Is it co-medication only? In case of relevant co-medication useful

Re-phrase: “by spiking increasing concentrations…” by “spiking with decreasing amounts of interfering molecules to detect the lowest level for acceptable interference”

Include sentence to allow for a Risk based approach for specificity testing

Remaining questions for panel discussion

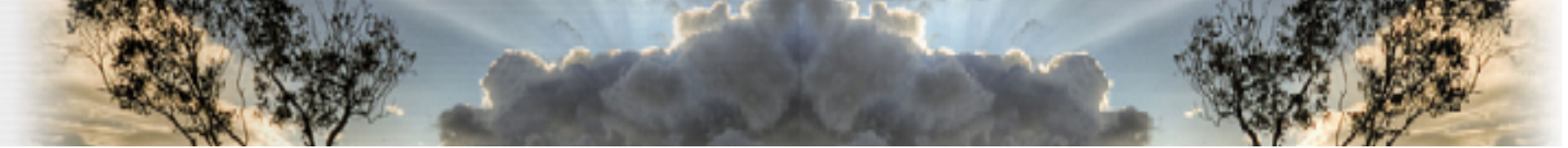
- Questions to the audience from the presenter

  Specificity is evaluated by spiking blank matrix samples with related molecules at the maximal concentration(s) of the **structurally related molecule anticipated in study sample**s.

  During method development and early assay validation, these **"related molecules" are frequently not available**. **Additional evaluation** of specificity may be **conducted after the original validation is completed**.

  - Does this answer when and how many?

  How to capture best "wanted interference" in a sentence?



4.2.2 Selectivity

4.2.2 Selectivity

Selectivity is the ability of the method to detect and differentiate the analyte of interest in the presence of other “unrelated compounds” (**non-specific interference) in the sample matrix**. The matrix can contain non-specific matrix component such as degrading enzymes, heterophilic antibodies or rheumatoid factor which may interfere with the analyte of interest.

Selectivity should be evaluated at the low end of an assay where problems occur in most cases, but it is recommended that selectivity is also evaluated at higher analyte concentrations. Therefore, selectivity is evaluated using **blank samples** obtained **from at least 10 individual sources** and by spiking the individual blank **matrices at the LLOQ and at the high QC level**. The **response** of the blank samples should **be below the LLOQ in at least 80%** of the individual sources. The **accuracy** should be within **±25% at the LLOQ** and **within ±20% at the high QC** level of the nominal concentration **in at least 80%** of the individual sources evaluated.

4.2.2 Selectivity

Selectivity should be evaluated in **lipaemic samples and haemolysed samples** (Refer to Section 3.2.1). For lipaemic and haemolysed samples, tests can be **evaluated once using a single source of matrix**. Selectivity should be assessed in **samples from relevant patient populations**. In the case of relevant patient populations there should be **at least five individual patients**.

Selectivity: comparison to current guidelines

	ICH M10	EMA 2012	FDA 2018	MHLW 2014	ANVISA 2012
Sample Types	include lipaemic and haemolyzed (once; single source of matrix) Should be assessed in at least 5 individual relevant patient matrix	Include lipaemic and haemolyzed; strongly recommended relevant disease population	Appropriate biological matrix, haemolyzed, lipaemic and special populations depending on intended use	Blank samples	Normal +1 lipaemic +1 haemolyzed
Sample number	10 individual sources	10 individual sources	10 individual sources	10 individual sources	nd
Spike Levels	Unspiked and LLOQ + HQC	Unspiked and spiked at/near LLOQ	Unspiked and LLOQ + HQC	Unspiked and at/near LLOQ	nd
Acceptance Criteria	Unspiked: ≥80% BQL Accuracy within ±25% at LLOQ and ±20% at HQC in ≥80% of matrices tested	Accuracy within ±20% (±25% at LLOQ) in ≥80% of matrices tested	Unspiked: ≥80% BQL Accuracy within ±25% at LLOQ and ±20% at HQC in ≥80% of matrices tested	Unspiked: ≥80% BQL Accuracy within ±20% near LLOQ or ±25% at LLOQ in ≥80% of matrices tested	nd

EBF position on the subject based on EBF-IGM Survey 2017 presented in Lisbon-2017

- EBF recommendation for minimum requirements for Selectivity as validation parameter:
  - At least 10 individual sources of HV and relevant matrix
  - No routine test of lipaemic and haemolysed samples
  - Spike level at or near LLOQ (including a definition of near)
  - 80% of tested blank sources BQL
  - 80% of spiked sources within 25% at or near LLOQ (within 2x LLOQ)

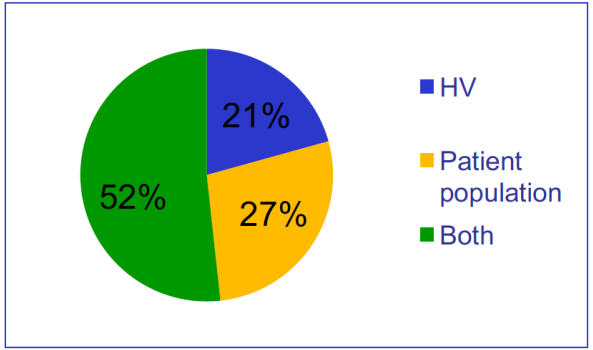
Impact on our industry – value for industry

- Selectivity is in line with *Current Best Practice*
- the inclusion of testing at HQC is copied from FDA guideline 2018
- Ambiguity of near LLOQ replaced by at LLOQ
- Asked for routine testing of lipaemic and heamolysed samples
- Number of relevant patient population samples to be tested is defined with n≥5

Current Best Practice:
EBF-IGM Survey 2017 presented in Lisbon-2017

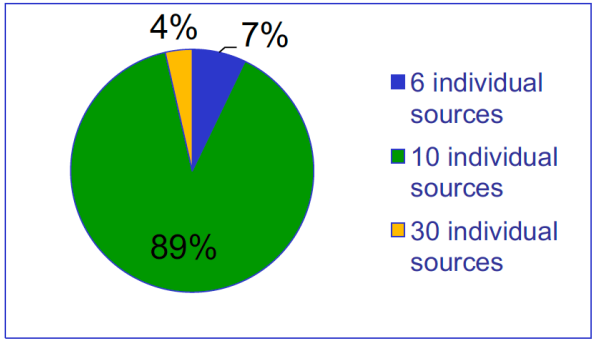
Individual Sources:

- What sources do you use:



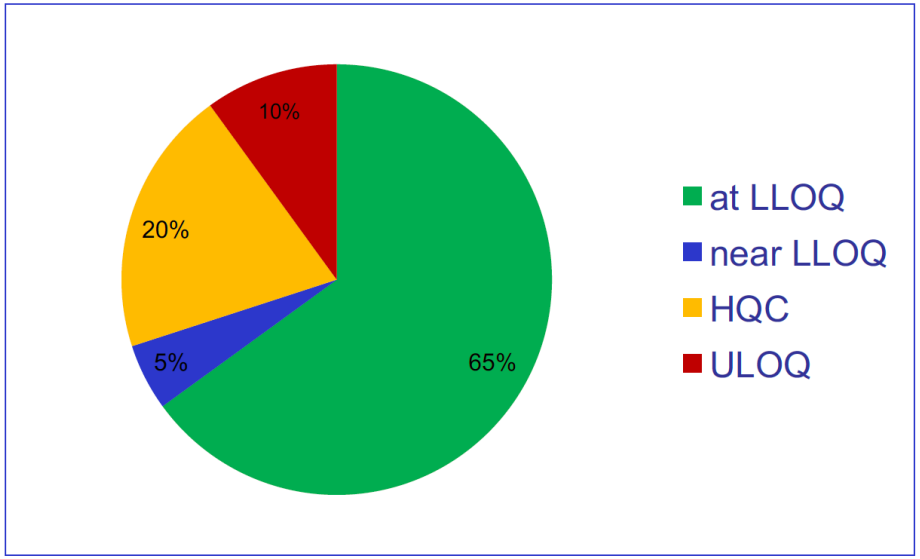
HV = Healthy Volunteers

- How many individual sources do you use:



Current Best Practice:
EBF-IGM Survey 2017 presented in Lisbon-2017

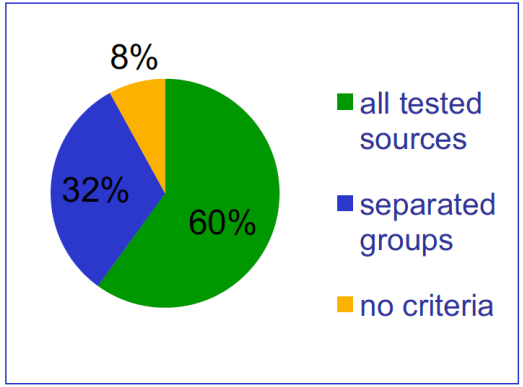
Distribution of spike levels:



Current Best Practice:
EBF-IGM Survey 2017 presented in Lisbon-2017

Selectivity Acceptance Criteria

- Unspiked:
  - ✓ ≥80% of samples tested ≤ LLOQ; for all tested individual sources (HV, patient population, lipemic / haemolysed)
  - ✓ ≥80% of samples tested ≤ LLOQ; for all groups evaluated separately
  - ✓ No criteria for unspiked sources



- Spiked (100% of responses for the same acceptance criteria):

  ≥80% of samples ≤ ±20% of nominal (≤ ±25% at LLOQ)

Impact on our industry – value for industry

- Selectivity is in line with *Current Best Practice*
- the inclusion of testing at HQC is copied from FDA guideline 2018
- Ambiguity of near LLOQ replaced by at LLOQ
- Asked for routine testing of lipaemic and heamolysed samples, but is part of the 10 individuals to be tested
- Number of relevant patient population samples to be tested is defined with n≥5

Feedback from EBF & Delegates

No harmonization between LBA and CC (10 vs 6 individual sources) in ICH M10. It looks like this difference is based on statistics covering the difference in acceptance criteria (20% vs 15%)

Inconsistency between Selectivity and Specificity: HQC vs ULOQ
(suggestion: LLOQ and one additional level)

Rewrite what is specified for chromatography regarding lipaemic samples

To be read as 10 or 10+1+1?

Lipaemic/haemolysed samples should not necessary for non-clinical, acceptance criteria? Should not be routinely evaluated as a checklist exercise?

Specify the minimum of triglyceride levels (possible specification 300mg/dl if lipid metabolism is not affected)

Single source of matrix = one pool?

Selectivity should be assessed in samples from relevant patient populations. In the case of relevant patient populations there should be at least five individual patients.
First "relevant patient population" means given study population second "relevant patient population" means patient population where Selectivity is known to be critical

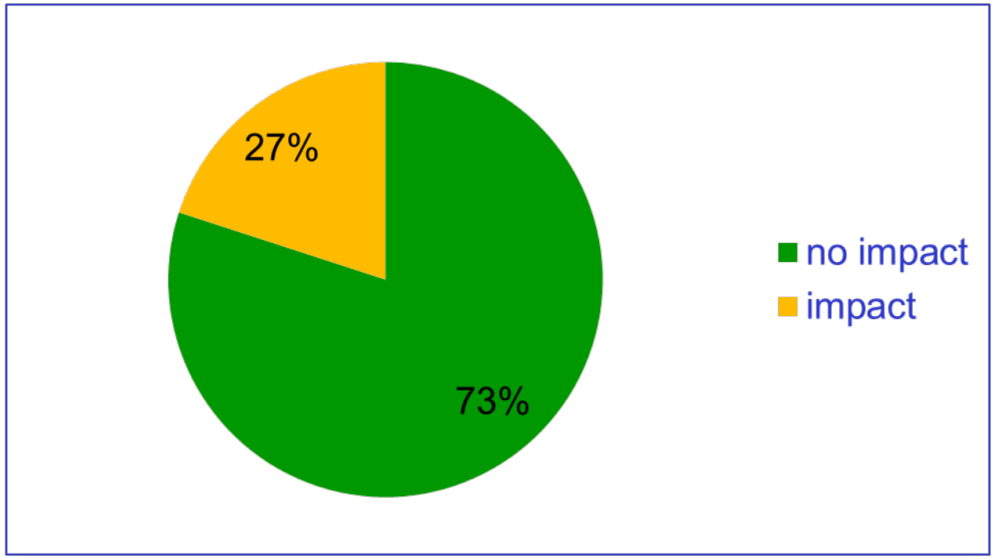
Procedure if no relevant patient material is available for validation is not described (use of pre-dose samples?)

AAPS survey - EBF members answers

Survey (Sept2017):

Lipemic & Haemolysed (n=15)

What is your experience with Lipemic & Haemolysed samples:



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
- Further questions to info@e-b-f.eu before 31 May