Selectivity and interference challenges for LBA

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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

- Mix of definitions:
  Selectivity/ Specificity/ Matrix effect
- Differences in current local guidelines
  => Current best practice within EBF IGM community
- Is MRD a validation parameter?

Selectivity vs Specificity

… are dependent on reagent and patient biology!

	Selectivity	Specificity
Definition	Ability of the method to detect and differentiate analyte of interest in the presence of other/ "unrelated compounds" in the sample	Ability of the method to detect and differentiate the analyte of interest in the presence of other/ "structurally related compounds" in the sample
Examples	• Enzymes • Reumathoid factor • Con-comitant small molecule	• Endogenous molecules • Related molecules

Selectivity *vs* Specificity

… are dependent on reagent and patient biology!

	Selectivity	Specificity
Issue	Lack of Selectivity can result in inhibition or enhancement of the signal. In general signal suppression from binding proteins occur more often	Lack of Specificity often leads to false positive and/or overestimation of analyte concentration
		Often not available at the time of first validation

Selectivity
What is in the Guidance/Guidelines (I)

	EMA 2012	MHLW 2014 (LBA)	ANVISA 2012	FDA (Draft) 2013
Definition	Ability to measure the analyte of interest in the presence of unrelated compounds in the matrix	Ability to detect and differentiate the analyte in the presence of other components in the samples	No specific tests for LBA. Allows for adaptations	Ability of an analytical method to differentiate and quantify the analyte of interest in the presence of other components in the sample.
				Evaluate concomitant medications (+ metabolites), cross-reactivity due to endogenous compounds
				Compare the LBA to a "validated reference method" such as LCMS using incurred samples
				Parallelism - diluting study samples with diluted standards to understand matrix effects
Sample Types	…include lipemic and haemolysed samples, relevant disease population	Not defined	Normal +1 hyperlipidaemic + 1 haemolysed (SM)	Not defined

Selectivity
What is in the Guidance/Guidelines (II)

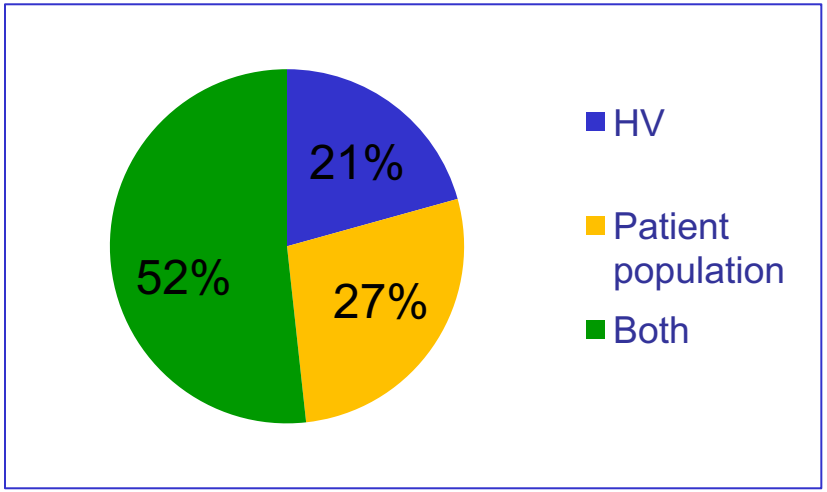
	EMA 2011	MHLW 2013	ANVISA 2012	FDA (Draft) 2013
Sample number	10 individual sources (incl lipemic and haemolysed)	10 individual sources	Not defined	At least 6 different sources
Spike Levels	Unspiked and spiked at/near LLOQ. When interference is conc dependent…determine the min conc where interference occur	Unspiked and spiked at/near LLOQ.	Not defined	Unspiked and spiked at/near LLOQ. Evaluate matrix effects using standard curve in matrix and compare to buffer curve using at least 10 sources of blank matrix
Acceptance Criteria	80% of the blank samples below LLOQ.	80% of the blank samples below LLOQ.	Not defined	Not defined
	Accuracy within ±20% (25%) near LLOQ (at LLOQ) in at least 80% of samples	Accuracy within ±20% (25%) near LLOQ (at LLOQ) in at least 80% of samples	Not defined	Not defined

EBF members current practice

EBF-IGM Survey (Sept 2017 / N=29)

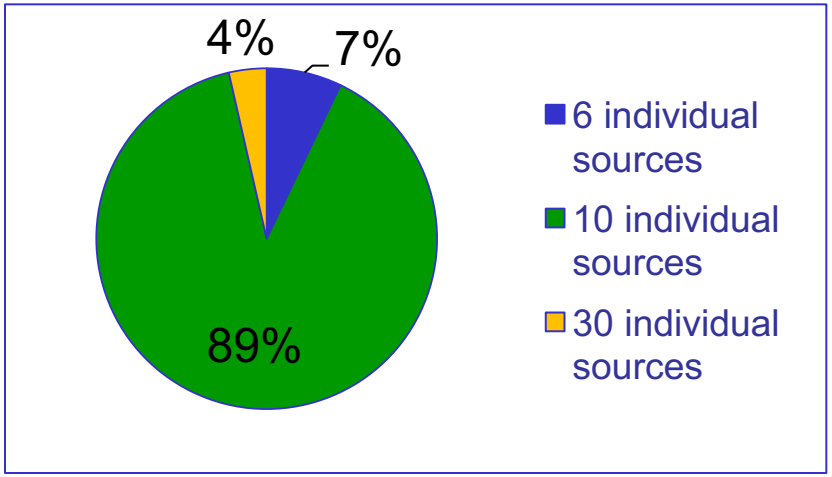
Individual Sources:

- What sources do you use:



HV = Healthy Volunteers

- How many individual sources do you use:



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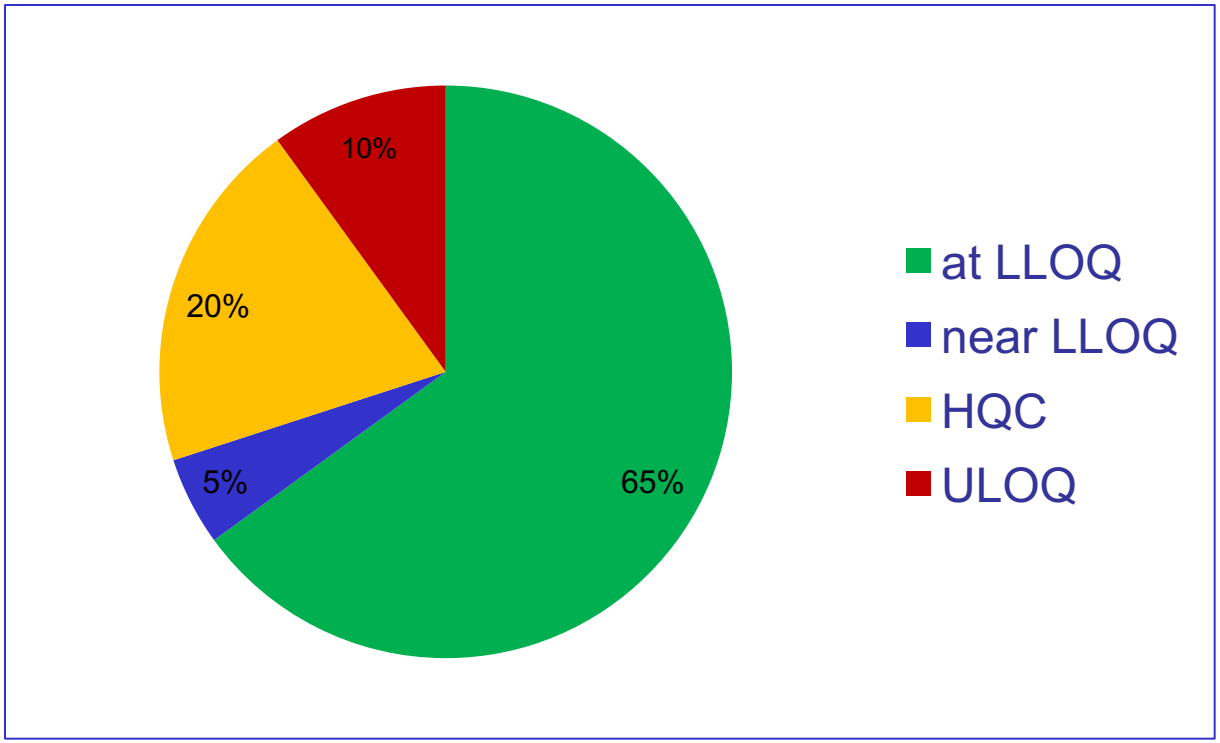
Distribution of spike levels:



EBF members current practice

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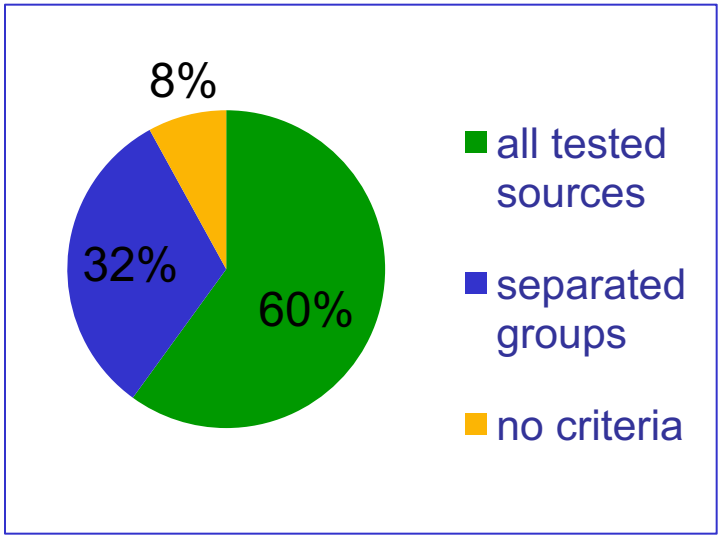
Selectivity Acceptance Criteria

- 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)

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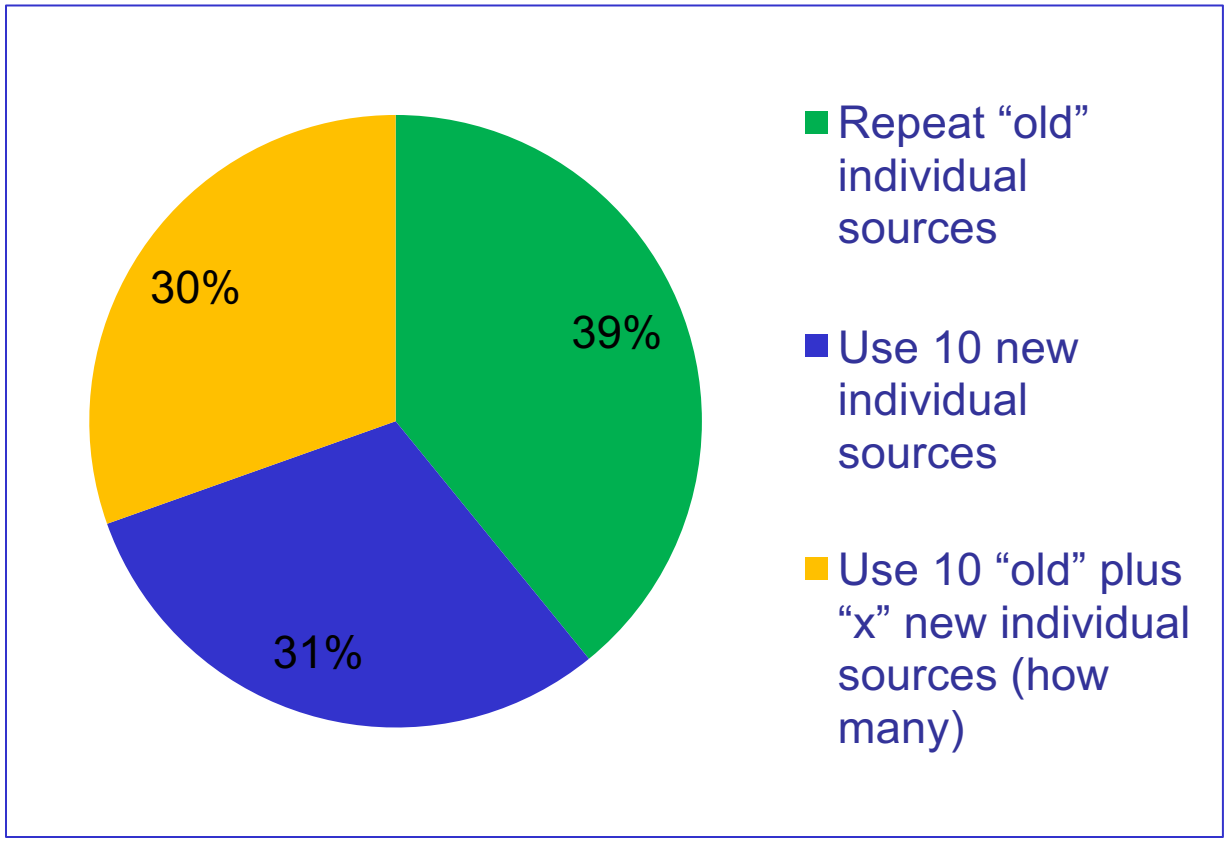
If Selectivity fails…



… case-by-case

Selectivity: Current Best Practice

- 10 or more individual lots of HV & relevant disease indication (if available).
  - Unspiked
  - Spiked at the LLOQ / “near” LLOQ (define “near” i.e. 2-3xLLOQ)
- Acceptance Criteria
  - ≥80% of the unspiked samples should measure ≤LLOQ
  - ≥80% of the spiked samples should be within ± 20% of nominal (± 25% at LLOQ)
  - The same 80% (or more) samples should meet criteria at both levels
- If appropriate disease state is not available, consider in-study selectivity assessment using pre-dosed samples

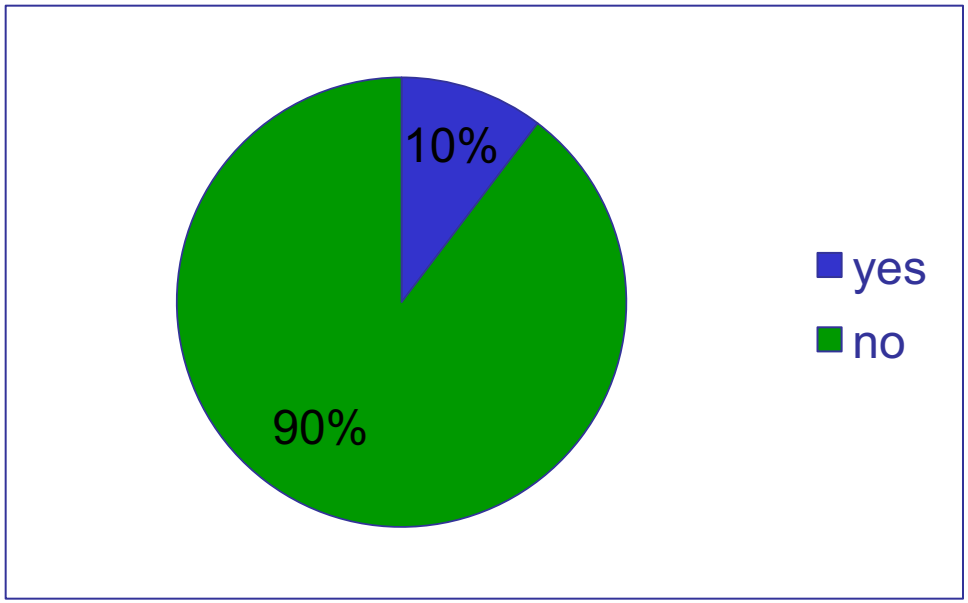
EBF members current practice

EBF-IGM Survey (Sept 2017 / N=29)

MRD

PK assay calibrators are typically in matrix – extensively addressed during method development to set up the right method and fix the MRD

=> Do you repeat MRD within Validation?

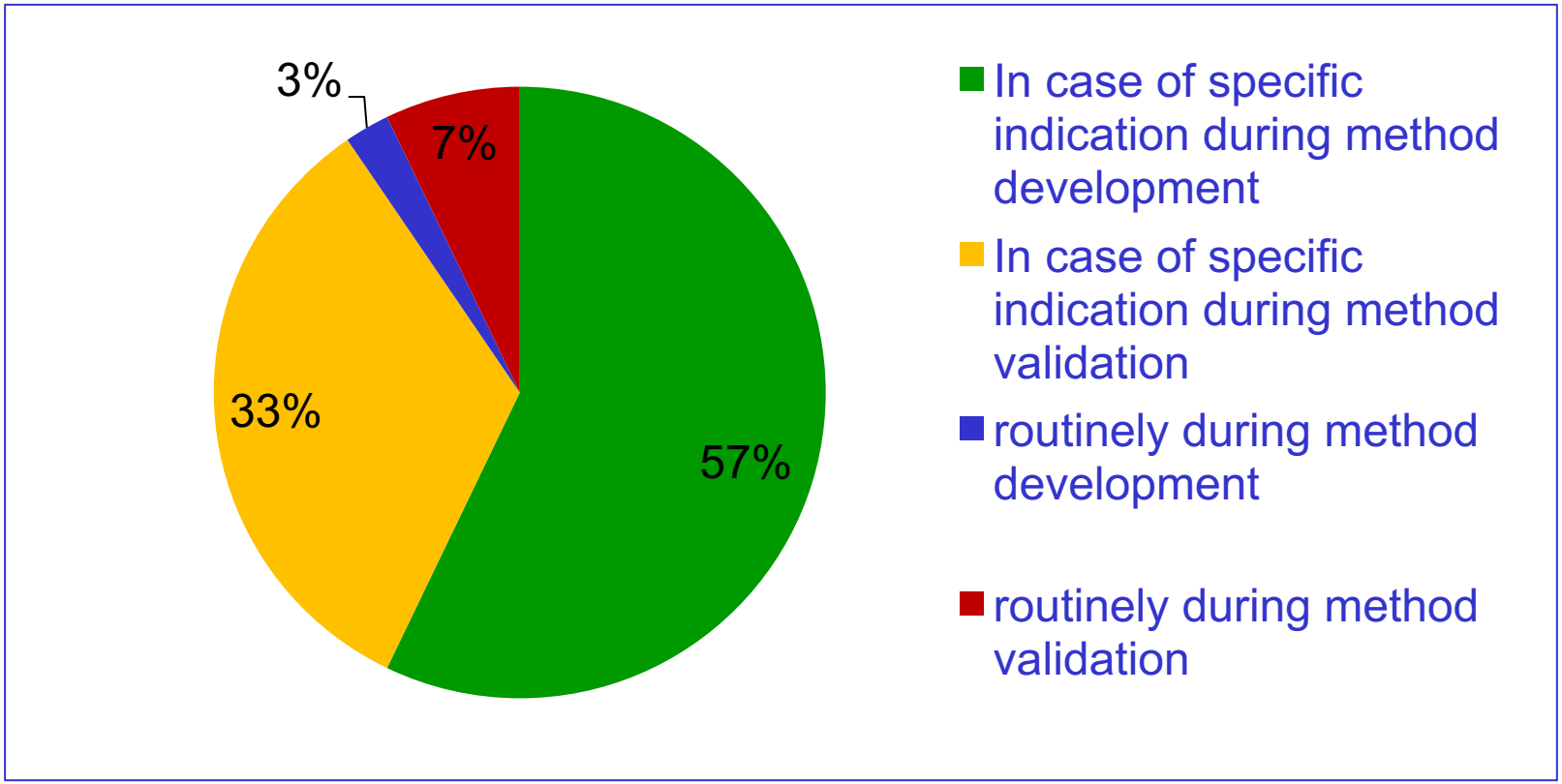


EBF members current practice

EBF-IGM Survey (Sept 2017 / N=29)

Interference testing

Specific interferences testing (i.e. degrading enzymes, RF,…)

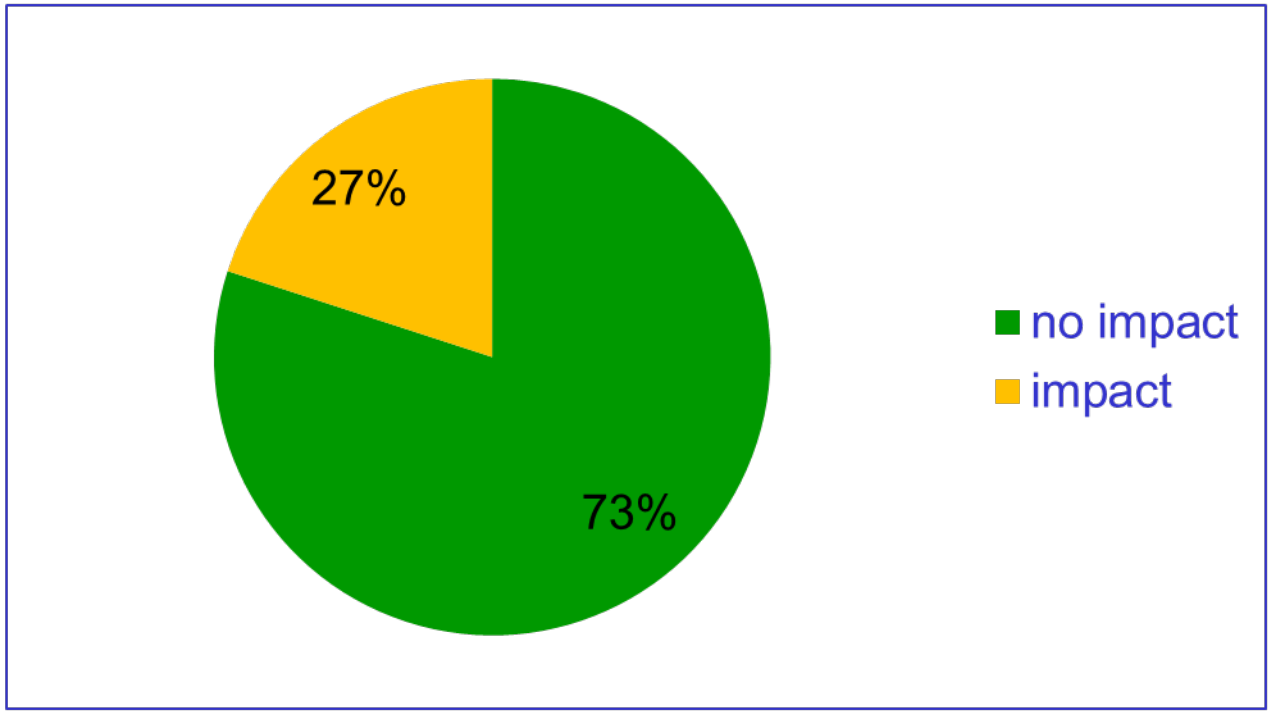


AAPS survey - EBF members answers

Survey (Sept2017):

Lipemic & Haemolysed (n=15)

What is your experience with Lipemic & Haemolysed samples:



Recommendation in the focus of ICH M10

1. Harmonized Definitions regarding guidelines but also between Chromatographic and LBA
2. MRD/ interference is the basis of an LBA therefore an extensive part of MD not MV (Reported in MV Report)
3. Min. requirements for Selectivity as validation parameter
   - At least 10 individual sources of HV and relevant matrix (no routine test of lipemic & haemolysed samples)
   - Spike level: LLOQ or near LLOQ (define near)
   - Acceptance
     - 80% of tested blank sources <LLOQ
     - 80% of spiked sources (at LLOQ) within 25%

Acknowledgement

- EBF team of this session “Hot topics in LBA“
- EBF IGM core members
- EBF community

It‘s a big challenge, but….

Never give up

…on the way to globally harmonised BMV criteria

References

- **EBF discussions and surveys**
- **GBC L2 team recommendations**
  Stevenson et al, AAPS Journal, 16(1), 2014
- **Crystal City V discussions**
- **AAPS ICHM10 Workshop Weehawken Sept2017**
- **Recommendations for the Bioanalytical Method Validation of Ligand-binding Assays to Support Pharmacokinetic Assessments of Macromolecules**
  DeSilva et al, Pharm Res, 20(11), 2003
- **Comparative assessment of bioanalytical method validation guidelines for pharmaceutical industry**
  Kadian et al, Journal of Pharmaceutical and Biomedical Analysis126, 2016
- **Crystal City V workshop report**
  Booth et al, AAPS Journal, 17(2), 2015

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