



## **Industry Feedback on ICH M10**

BioBridges 2019

Philip Timmerman (EBF)

# This presentation

1. EBF
2. EBF and ICH M10
  - Consolidating industry FB
  - Considering the world around us
  - Some highlights from the industry FB
    - Background and scope
    - A few Stability paragraphs
    - Method development requirements
    - Documentation requirements
    - Incurred Sample Reanalysis
    - Partial and cross validation

- **Founded in 2006** (Non for profit organisation)
- **Currently 67 member companies** from Pharma & CRO → R&D based companies, active in regulated bioanalysis in Europe
- **Operating structure**
  - Steering Committee (n=3) + chairman (n=1)
  - Core member community: Assigned by company 1 “core” representative for small molecules 1 for large molecules
  - Project teams: managed by team leads and SC
- **Internal meetings, n=2**
  - Strategy Meeting and General Assembly, March, Belgium
  - YEMM, connected to Open Symposium
- **External meetings, n=4**
  - YSS - March, Bologna
  - Spring FW = May, Malaga/Lisbon
  - Autumn FW = September, Lisbon/Malaga, Spain
  - Open Symposium = November, Barcelona, Spain
- EBF only represents EBF and not individual member companies
- Within EBF, we don't :
  - Exchange portfolio or IP information
  - Allow advertisements of members or invites
  - Engage in business development
  - Misuse the EBF brand and logo.
- Engagement in single vendor relationships is strictly limited and needs approval by the steering committee
- EBF will not present at a ‘purely for profit’ meeting

# EBF Mission

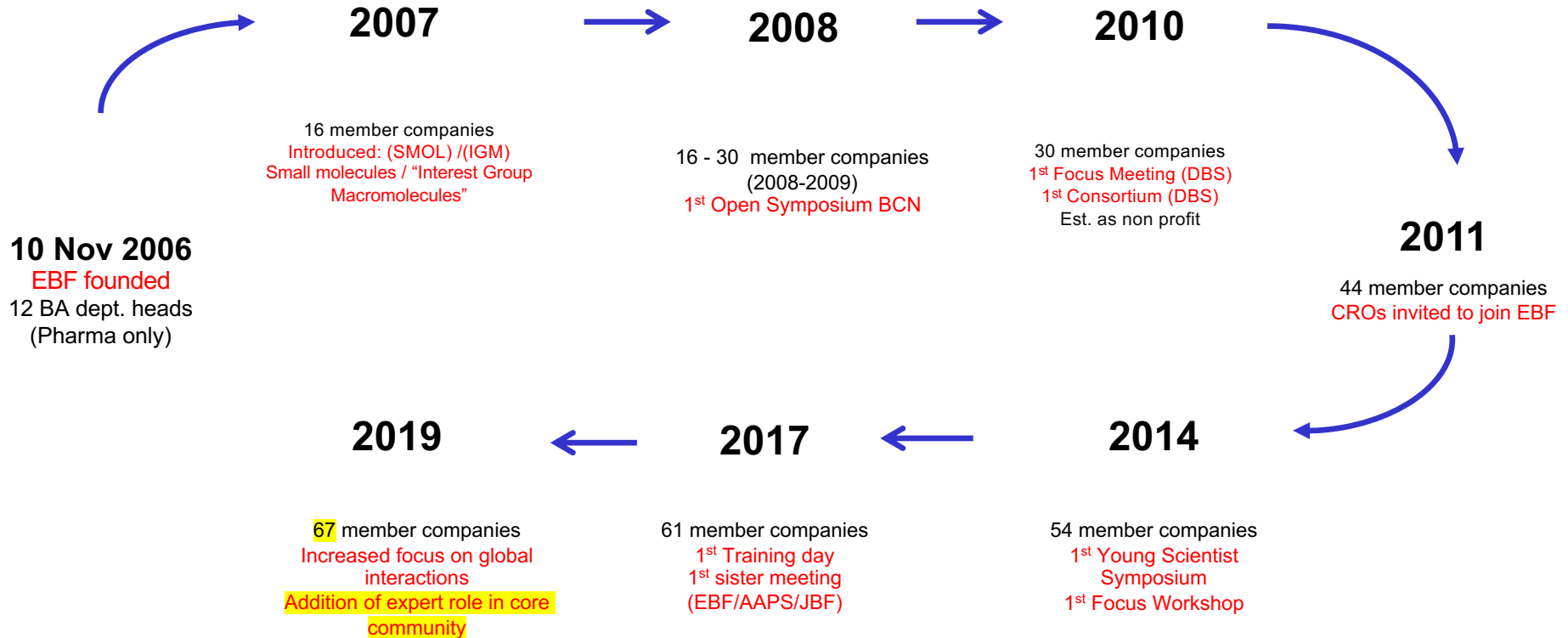
Our mission is to share, discuss, optimize and seek alignment on a broad array of bioanalytical topics including science, procedures, business tools and technology, and regulatory issues.

Internal discussions within EBF aim to recommend or influence opinions/procedures towards our members, business partners, regulatory bodies and any other stakeholders.

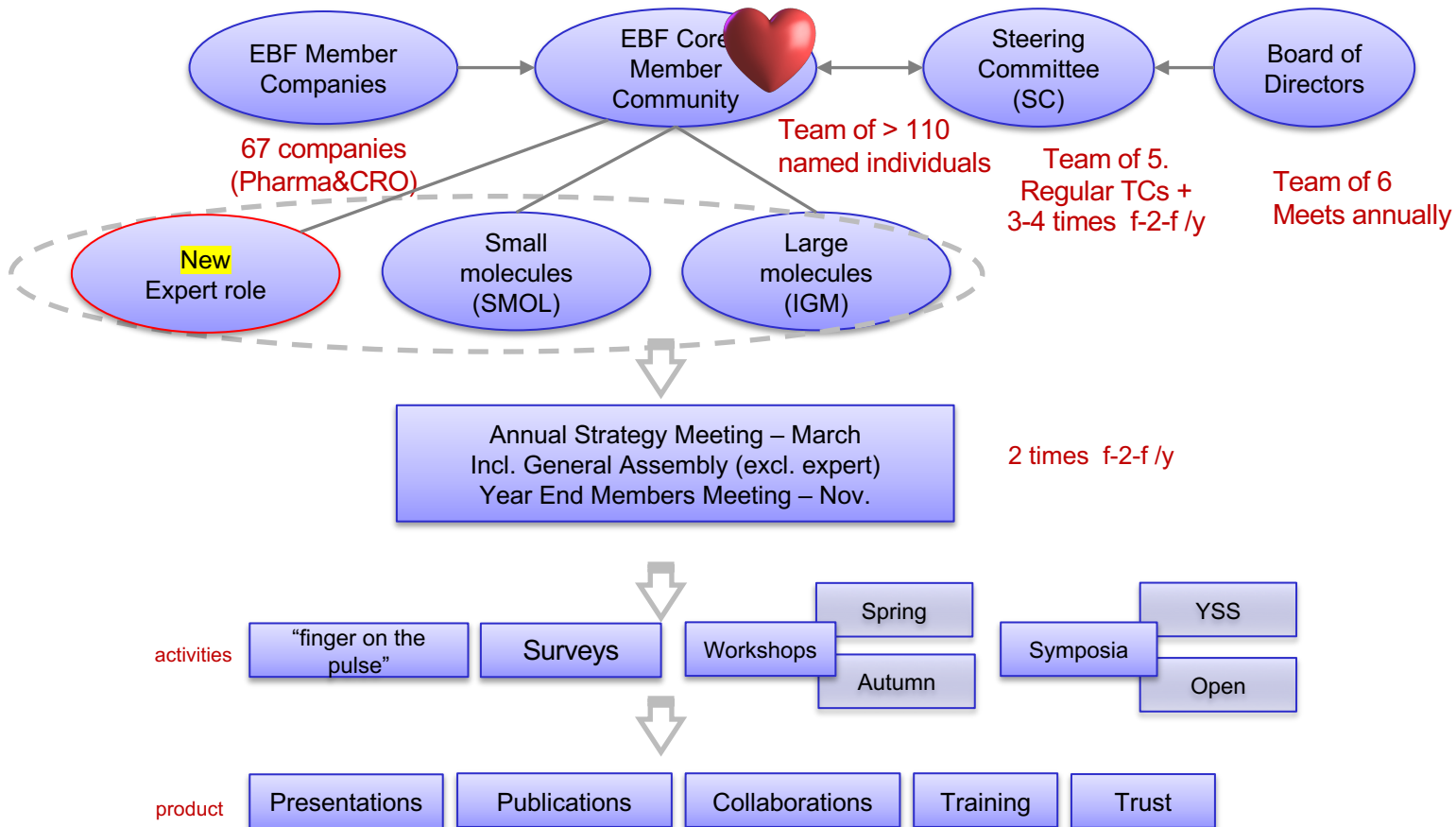
Going forward, EBF is providing guidance and recommendations to the European and Global bioanalytical community.

Finally, provide development opportunities for EU based scientist by joining cross company collaborations and contributions to peer reviewed journals, international meetings and symposia.

# A Brief History of the EBF

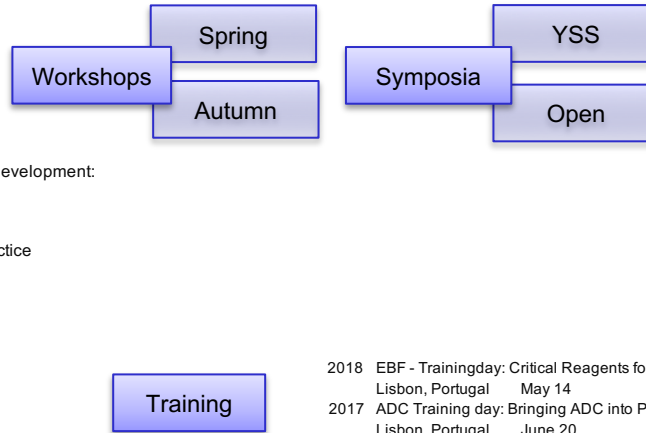


# EBF Operating Model



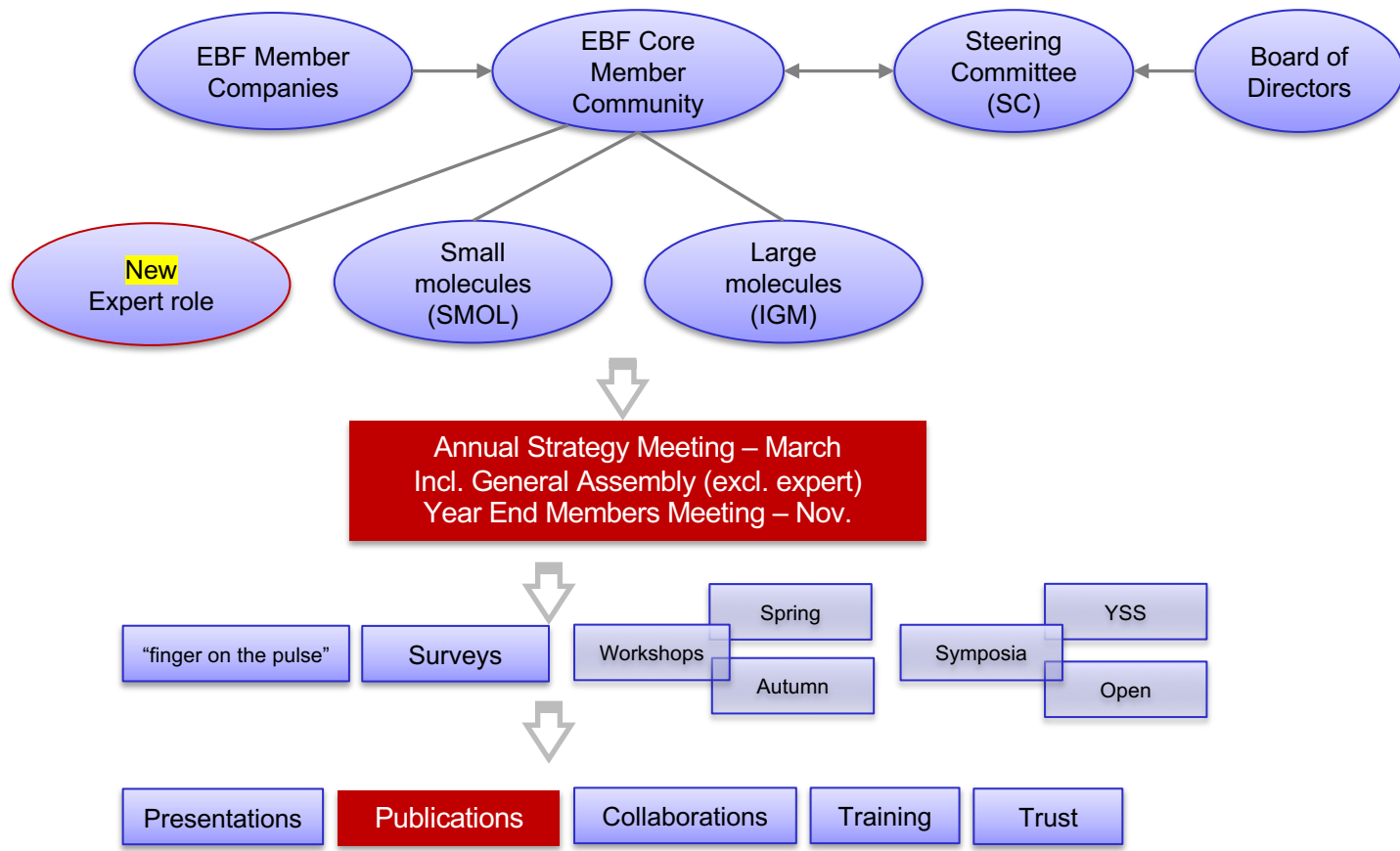
- 2018 Today's challenges and solutions in assessing immunogenicity in patients  
Lisbon, Portugal September 19-20
- 2017 Industry input into ICH M10: Experimental data as the cornerstone for a science driven bioanalytical guideline  
Lisbon, Portugal September 24-26
- 2016 Current analysis of immunogenicity – Best Practices and Regulatory Hurdles  
Lisbon, Portugal September 27-28
- 2015 The 'W4' of Metabolite profiling and quantification strategies in drug R&D: When, What, Why and Who?  
Brussels, Belgium September 25
- 2014 China Days – Meet the Dragon  
Berlin, Germany, September 11-12

- 2018 New Modalities and Novel Concepts in Bioanalysis  
Lisbon, Portugal May 15-16
- 2018 EBF - Trainingday: Critical Reagents for LBA  
Lisbon, Portugal May 14
- 2017 Bioanalytical Strategies for Large Molecules in Modern Drug Development: LBA and LC-MS united  
Lisbon, Portugal June 21-22
- 2016 Bringing Assay Validation and Analysis of Biomarkers into Practice  
Lisbon, Portugal June 9-10
- 2015 Optimizing the Pharma CRO scientific interface in bioanalysis  
Brussels, Belgium, March 12-13
- 2012 Hatching  
Brussels, Belgium June 12-13
- 2011 Large meets Small  
Brussels, Belgium June 21-22
- 2010 Connecting Strategies on Dried Blood Spots  
Brussels, Belgium June 17-18



- 4th The BioA Brain: Embracing new ideas  
Ghent, Belgium March 15-16, 2018
- 3rd In Unity Lies Power; Building a Better Bioanalytical World Together  
Barcelona, Spain November 15, 2016
- 2nd Future of Bioanalysis... A Bridge between Industry and Academia  
Barcelona, Spain November 17, 2015
- 1st Unleashing the Future  
Barcelona, Spain November 18, 2014

- 10th 10 – A New Journey begins  
Barcelona, Spain November 15-17, 2017
- 9th Reaching Utopia – The Kaleidoscope of Bioanalysis  
Barcelona, Spain November 16-18, 2016
- 8th Into New Territories – Explore, Learn and Apply  
Barcelona, Spain November 18-20, 2015
- 7th Beyond the Horizon – Painting a new landscape  
Barcelona, Spain November 19-21, 2014
- 6th Moving Forward Together  
Barcelona, Spain November 20-22, 2013
- 5th Old Battles and New Horizons  
Barcelona, Spain November 14-16, 2012
- 4th Less is More  
Barcelona, Spain November 16-18, 2011
- 3rd From Challenges to Solutions  
Barcelona, Spain December 1-3, 2010
- 2nd The Broadening Scope of Validation  
Barcelona, Spain December 2-4, 2009
- 1st Burning issues in Bioanalysis  
Barcelona, Spain December 1-2, 2008





## Some Recommendation papers

**Incurred sample reproducibility:** views and recommendations by the European Bioanalysis Forum,

Best practices in a tiered approach to **metabolite quantification:** views and recommendations of the European Bioanalysis Forum;

EBF recommendation on the validation of bioanalytical methods for **dried blood spots;** Bioanalysis, Vol. 3, No. 14, Pages 1567-1575.

**Anticoagulant counter ion** impact on bioanalytical LC-MS/MS assay performance: additional validation required?

European Bioanalysis Forum recommendation: scientific validation of quantification by **accelerator mass spectrometry;**

Bioanalysis for **plasma protein binding studies** in drug discovery and drug development: views and recommendations of the European Bioanalysis Forum

EBF recommendation for **stability testing of anti-drug antibodies;** lessons learned from anti-vaccine antibody stability

Recommendations from the European Bioanalysis Forum on method establishment for **tissue homogenates.**

Tiered approach into practice - **scientific validation for chromatography-based assays:** a recommendation from the European Bioanalysis Forum,

> 20 recommendation papers

## Some Strategic papers

Development of a **Generic Laboratory Manual** for Biological Sample Logistics in Clinical Pharmacokinetic Studies

Towards **decision-based acceptance criteria** for Bioanalytical Method Validation: a proposal for discussion from the EBF

Feedback from the European Bioanalysis Forum Workshop: **Taking tiered approach to the next level.**

How the bioanalytical scientist plays a key role in **interdisciplinary project teams** in the development of biotherapeutics – a reflection of the European Bioanalysis Forum

LC-MS/MS of large molecules in a regulated bioanalytical environment – **which acceptance criteria to apply?**

Managing scientific, technical and regulatory innovation in regulated bioanalysis: a discussion paper from the European Bioanalysis Forum

Global Bioanalysis Consortium - Working towards a functional globally acceptable and **harmonized guideline on bioanalytical method validation**

**Request for Global Harmonization** of the Guidance for Bioanalytical Method Validation and Sample Analysis

European Bioanalysis Forum recommendation on **method establishment and bioanalysis of biomarkers** in support of drug development

> 20 strategic papers

## Disclaimer

*The FB on ICH M10 in this presentation was prepared on behalf of EBF, incorporating to the best of our ability the outcome of internal EBF discussions, - surveys, discussions from the EBF Barcelona Industry Focus Workshop (sister meeting) and from EFPIA discussions.*

*The opinions expressed in this presentation do not necessarily reflect the view of any individual expert, EBF or EFPIA member company nor that of the ICH M10 Expert Working Group (EWG).*

## 2. EBF and ICH M10

- a) Consolidating industry FB
- b) Considering the world around us
- c) Some highlights from the industry FB during public consultation
  - Background and scope
  - A few Stability paragraphs
  - Method development requirements
  - Documentation requirements
  - Incurred Sample Reanalysis
  - Partial and cross validation

## 2. a. Consolidating industry FB

EBF presentations are consolidated community opinions.

Input gathered from:

- Internal EBF surveys:
- comments on ICH M10 Guideline (March 2019) – 45 companies provided written FB, 65 companies participated in pre-FW discussions during an internal EBF face-to-face meeting
- Gauge for Agreement/ambiguity/disagreement with paragraphs
- Include legacy EBF recommendations on relevant BMV sections – publications available in Bioanalysis Journal

All meeting delegates were invited fill out a similar survey:

- We received written comments from 21 companies.
- All presenters were asked to integrate comments from delegates in their presentations.

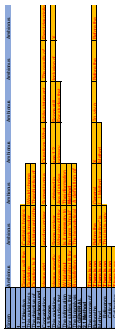
**> 1.100 individual comments**

I feel this paragraph is ambiguous

Green	Ambiguous	Ambiguous	Ambiguous	Ambiguous	Ambiguous	Ambiguous	Ambiguous
<b>1</b>							
1.1 Objective	General: will the	Certain topics					
This guideline is	Guideline	Needs greater	Would change				
The objective of	Question: Is it	A statement in the	The objective of				
<b>1.2 Background</b>							
Concentration	please add	wording unclear	Suggest some	Questions: What	while statement	The results of	Exact descriptio
<b>1.3 Scope</b>							
This guideline	please specify	Scope unclear	obtained in	see 1.2	Scope still	Inclusion of	X
For studies that	propose scope	What about fit for	Are all studies	primary	For studies that		
The information	What about	Is it necessary to	The information				
For studies that	What about fit for	the term 'should'	Method				
The bioanalysis	By definition a	The bioanalytical	A definition of				
<b>2 GENERAL</b>							
<b>2.1 Method</b>							
The purpose of	There is no						
Before the	There is no	Before the	General	X	In vivo	Before the	Before the
Method	There is no	"extracting" is	1.1 Method	Method			
· Reference	There is no	This list is very					
· Critical	There is no						
· Calibration	There is no						

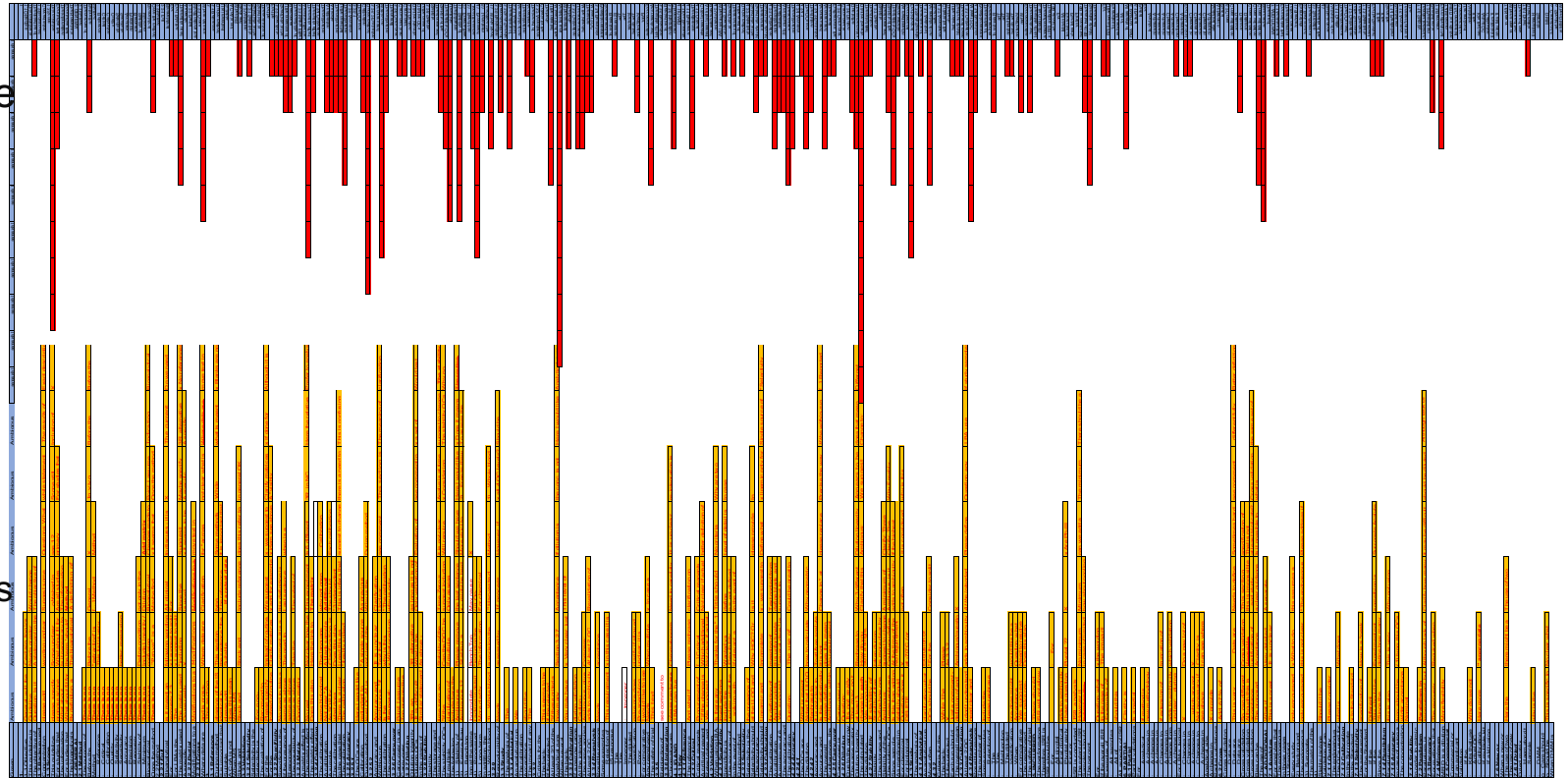
XXXXXXXXXX

I feel this paragraph is ambiguous



I disagree

I feel this paragraph is ambiguous



Ambiguous

<b>1. INTRODUCTION</b>			
1.1 Objective	6		6
1.2 Background	9		
<b>1.3 Scope</b>	<b>21</b>	<b>8</b>	<b>38</b>
<b>2. GENERAL PRINCIPLES</b>			
<b>2.1 Method Development</b>	<b>26</b>	<b>3</b>	<b>29</b>
2.2 Method Validation	1		1
2.2.1 Full Validation	21	4	25
2.2.2 Partial Validation	4		4
2.2.3 Cross Validation	8	4	12
<b>3. CHROMATOGRAPHY</b>			
3.1 Reference Standards	18	1	19
3.2 Validation			0
3.2.1 Selectivity	14	2	16
3.2.2 Specificity	12	6	18
3.2.3 Matrix Effect	9	3	12
<b>3.2.4 Calibration Curve and Range</b>	<b>18</b>	<b>9</b>	<b>27</b>
3.2.5 Accuracy and Precision			
3.2.5.1 Preparation of Quality Control Samples	7	8	15
3.2.5.2 Evaluation of Accuracy and Precision	18	5	23
3.2.6 Carry-over	2	1	3
3.2.7 Dilution Integrity	12	4	16
<b>3.2.8 Stability</b>	<b>42</b>	<b>22</b>	<b>64</b>
3.2.9 Reinjection Reproducibility	6	2	8
3.3 Study Sample Analysis	1	3	4
3.3.1 Analytical Run	3	3	6
<b>3.3.2 Acceptance Criteria for an Analytical Run</b>	<b>13</b>	<b>13</b>	<b>26</b>
3.3.3 Calibration Range	7	6	13
3.3.4 Reanalysis of Study Samples	13	5	18
3.3.5 Reinjection of Study Samples	1	2	3
3.3.6 Integration of Chromatograms	5	2	7
<b>4. LIGAND BINDING ASSAYS</b>			
4.1 Key Reagents	1		1
4.1.1 Reference Standard	3	3	6

Ambiguous

4.1.2 Critical Reagents	9		9
4.2 Validation	5		5
4.2.1 Specificity	12	2	14
4.2.2 Selectivity	14		14
4.2.3 Calibration Curve and Range	11	8	19
4.2.4 Accuracy and Precision		6	6
4.2.4.1 Preparation of Quality Control Samples	4	3	7
4.2.4.2 Evaluation of Accuracy and Precision	18	2	20
4.2.5 Carry-over	1	2	3
<b>4.2.6 Dilution Linearity and Hook Effect</b>	<b>23</b>	<b>13</b>	<b>36</b>
<b>4.2.7 Stability</b>	<b>26</b>	<b>12</b>	<b>38</b>
4.3 Study Sample Analysis			
4.3.1 Analytical Run	5	3	8
4.3.2 Acceptance Criteria for an Analytical Run	7	2	9
4.3.3 Calibration Range	9	4	13
4.3.4 Reanalysis of Study Samples	11	10	21
<b>5. INCURRED SAMPLE REANALYSIS (ISR)</b>	<b>24</b>	<b>10</b>	<b>34</b>
<b>6. PARTIAL AND CROSS VALIDATION</b>			
6.1 Partial Validation	1	0	1
<b>6.2 Cross Validation</b>	<b>26</b>	<b>10</b>	<b>48</b>
<b>7. ADDITIONAL CONSIDERATIONS</b>			
7.1 Analytes that are also Endogenous Compounds	10	3	13
7.1.1 Quality Control Samples	3	1	4
7.1.2 Calibration Standards			0
7.1.3 Selectivity, Recovery and Matrix Effects	9	3	12
7.1.4 Parallelism	3		3
7.1.5 Accuracy and Precision	4		4
7.1.6 Stability			0
7.2 Parallelism	5		5
7.3 Recovery	2	2	4
7.4 Minimum Required Dilution	1		1
7.5 Commercial and Diagnostic Kits	3		3
7.6 New or Alternative Technologies	3		3
7.6.1 Dried Matrix Methods	3		3



## Some Common themes highlighted

- Geographical differences in implementation and execution
  - In some regions, discussions with HA are (historically) encouraged vs. other regions will implement ICH M10 as part of their law
  - Will this effect open scientific interaction in less straightforward programs?
- Training
  - Common ICH training program is essential: for industry, regulators and inspectors, for successful implementation of the guideline.
  - Involving interprofessional organisations desired
- Ambiguity in Guideline continues fueling the risk of ISRc
  - Again....need for training and communication

## 2. b. Considering the world around us

Acceptance criteria  
GCP considerations  
3Rs

## Acceptance criteria

Why do we continue to rely on technology based criteria to support PK decisions? The data support similar safety or efficacy decisions...

EBF would like the industry and HA to consider an open and science based discussion on the added value of integrating harmonized decision-based acceptance criteria for PK bioanalytical assays

In this way, we create a transparent platform to facilitate the use of new technologies in the toolbox of the regulated bioanalytical scientist

\* *Toward decision-based acceptance criteria for Bioanalytical Method Validation: a proposal for discussion from the European Bioanalysis Forum*  
*Bioanalysis* (2018), 10 (16), 1255-1259

# GCP considerations

- Adherence to GCP remains controversial and ambiguous in BA labs
- Challenges within the bioanalytical lab to be resolved through continuous improvement and advancement of relevant GCP processes and trainings

## EBF/EFPIA - recommendation to EMA/EWG

### 1.3 Scope - cntd

For studies that are subject to Good Laboratory Practice (GLP) or Good Clinical Practice (GCP) the bioanalysis of study samples should also conform to their requirements.

For studies that are subject to Good Laboratory Practice (GLP) the bioanalysis of study samples must also conform to its requirements. **In accordance with Good Clinical Practice (GCP), the bioanalysis of clinical study samples must be conducted as described by the study protocol and within the limits of the informed consent agreed to by study participants.**

## 3Rs

The EU based BA community feels a modern, science based guideline should consider animal welfare and review unnecessary use of animals:

- Replace
  - Surrogate matrix used when valid. E.g. Sample dilutions, Calibrators
- Reduce
  - Using smaller volumes of sample or matrix. E.g. consider less replicates in preclinical assays, Reduce requirement for non-serial sampling or satellite groups
- Refine
  - Microsampling to reduce stress

## 2. EBF and ICH M10

- a) Consolidating industry FB
- b) Considering the world around us
- c) **Some highlights from the industry FB**
  - Background and scope
  - A few Stability paragraphs
  - Method development requirements
  - Documentation requirements
  - Incurred Sample Reanalysis
  - Partial and cross validation

## 2.c. Some highlights from the industry FB Background and scope

# Different perspectives

Evaluating a registration  
file on HA desk

Building a registration file  
on a Pharma R&D desk



# The Regulator's perspective

## 1.2 Background

- Concentration measurements of chemical and biological drug(s) and their metabolite(s) in biological matrices are an important aspect of drug development. The results of **pivotal** nonclinical toxicokinetic (TK)/pharmacokinetic (PK) studies and of clinical trials, including comparative bioavailability/ bioequivalence (BA/BE) studies, are **used to make regulatory decisions regarding the safety and efficacy of drug products**. It is therefore critical that the bioanalytical methods used are well characterised, **appropriately validated** and documented in order to ensure reliable data to **support regulatory decisions**.

## 1.3 Scope

This guideline describes the method validation that is expected for bioanalytical assays **that are submitted to support regulatory submissions**. The guideline is applicable to the validation of bioanalytical methods used to measure concentrations of chemical and biological drug(s) and their metabolite(s) in biological samples (e.g., blood, plasma, serum, other body fluids or tissues) obtained in pivotal nonclinical TK/PK **studies that are used to make regulatory decisions and all phases of clinical trials in regulatory submissions**. Full method validation is expected for the primary matrix(ces) intended to support regulatory submissions. Additional matrices should be partially validated as necessary. The analytes that should be measured in nonclinical and clinical studies and the types of studies necessary to support a regulatory submission are described in other ICH and regional regulatory documents.

For studies that are not submitted for regulatory approval or not considered for regulatory decisions regarding safety, efficacy or labelling (e.g., exploratory investigations), applicants may decide on the level of qualification that supports their own internal decision making.

The information in this guideline applies to the quantitative analysis by ligand binding assays (LBAs) and chromatographic methods such as liquid chromatography (LC) or gas chromatography (GC), which are typically used in combination with mass spectrometry (MS) detection and occasionally with other detectors.

For studies that are subject to Good Laboratory Practice (GLP) or Good Clinical Practice (GCP) the bioanalysis of study samples should also conform to their requirements.

**The bioanalysis of biomarkers and bioanalytical methods used for the assessment of immunogenicity are not within the scope of this guideline.**

## The Regulator's perspective

When reviewing a file, it can be assumed it's clear which studies in the file are pivotal / used to make claims on safety and efficacy

- ...and in extension, which analytes, matrices were analysed and which methods were used

**Scope paragraph of M10 will likely do the job**

## The Industry's perspective

Our surveys confirms that, when developing a drug, we may not know which studies will end up in the file to become pivotal / used to make certain claims on safety and efficacy...and in extension, which analytes, matrices were analysed and which methods require validation

# Survey comments on “Scope”:

a few representative ones from the > 50 we received

Scope unclear, and why does ICH refer to regional documents that it is supposed to replace?

Exact description required which assays are meant. This guideline does e.g. not cover special requirements for free drug assays.

Concerned that tissues are in same class as plasma with regard to level of validation

Scope could be better define. At the moment everything appears to be in scope.

The scope of the guideline seems too broad.

Scope is too wide now that it includes nonclinical studies. Definition of pivotal is crucial. How do you know what pre-clinical studies are pivotal, especially PK? Why are all clinical studies included?

I would exclude early clinical studies (escalation/expansion phase) to be analyzed with fully validated method (mainly applicable for LBA): indeed, with new biologic formats, often MABEL approach is used meaning very low doses for starting. The range of quantitation is therefore not known and fixed. At this stage, a fit for purpose method validation seems more appropriate.

# Tree, gorilla, Lion or fish? Let's find out...

- A list of typical studies that are submitted for analysis covering all areas of development and all analytes and matrices
- separate list for LBA and CHROM
- A simple 1 or 0 if study/analyte was thought to be in scope...
  - 2<sup>nd</sup> question asked: what do you desire should be in scope

## CHROMATOGRAPHY

## LBA

		plasma is the primary matrix				urine is a secondary matrix				tissues are a secondary matrix			
		plasma	plasma	plasma	plasma	urine	urine	urine	urine	tissue	tissue	tissue	tissue
matrix-->													
Analyte-->		dosed drug	M- active*	M > 10%*	M-not active	dosed drug	M- active*	M > 10%*	M-not active	dosed drug	M- active*	M > 10%*	M-not active
study	additional info on study												
pre-phase 1	NC	dose range study	rat	non GLP study									
pre-phase 1	NC	rat /dog/... PK		a non GLP PK study around the start of GLP									
pre-phase 1	NC	28d GLP		the first GLP study									
Phase 1	NC	6m GLP		any GLP study typically in a later drug development stage									
>phase 1	NC	mechanistic PK/TK		a non standard nonGLP study in a later drug dev. stage									
all phases	NC/Clin	PPB study		plasma protein binding studie using spiked samples only									
>Phase 1	Clin	PPB study		plasma protein binding studie using patient samples									
Phase 1	Clin	FIM - HV		First into Man study									
Phase 2a	Clin	FIM - onco		First into Man is often also First into Patient study in onco									
Phase 2a	Clin	FIP (start Ph-2)											
Phase 3-4	Clin	Patient studies		Any Patient study in > phase 2									
phase 1 ->4	Clin	food effect, BA,...		typical "non-BE" clin. study looking at (relative) exposures									
phase 1 ->4	Clin	DDI		This one can include looking at impact on (active?) metab.									
Phase 3-4	Clin	BE		Any BioEQ study									

		Serum		urine	tissue
		primary assay*	secondary assay*		
matrix-->					
Analyte-->					
study	additional info on study				
pre-phase 1	NC	dose range study	rat	non GLP study	
pre-phase 1	NC	rat /dog/... PK		a non GLP PK study around the start of GLP	
pre-phase 1	NC	28d GLP		the first GLP study	
Phase 1	NC	6m GLP		any GLP study typically in a later drug development stage	
> phase 1	NC	mechanistic PK/TK		a on standard nonGLP study in a later drug development stage	
Phase 1	Clin	FIM - HV		First into Man study	
Phase 2a	Clin	FIM - onco		First into Man is often also First into Patient study in onco	
Phase 2a	Clin	FIP (start Ph-2)			
Phase 3-4	Clin	Patient studies		Any Patient study in > phase 2	
phase 1 ->4	Clin	food effect, BA,...		typical "non-BE" clinical study looking at (relative) exposures	
phase 1 ->4	Clin	DDI		This one can include looking at impact on (active?) metabolites	
Phase 3-4	Clin	BE		Any BioEQ study	

# Observation on “Intended scope”



1 out of 4 or more have a different view on “in or out of scope”



1 out of 5 ...

## INTENDED SCOPE

			matrix --->				matrix --->				matrix --->			
			plasma		urine		tissue		plasma		urine		tissue	
			dosed	M-	M >	M >	M-	M >	dosed	M-	M >	M >	M-	M >
			drug	active*	10%*	active	drug	active*	10%*	active	drug	active*	10%*	active
study		additional info on study												
pre-phase 1	NC	dose range study rat	0	0		0	0	0	0	0	0	0	0	0
pre-phase 1	NC	rat/dog/... PK	13	13		0	13	13	0	8	8		0	0
pre-phase 1	NC	28d GLP	96	88		13	38	33	0	25	17		0	0
Phase 1	NC	6m GLP	100	100		17	38	38	0	25	21		0	0
>phase 1	NC	mechanistic PK/TK	21	21		4	21	21	4	17	17		4	4
all phases	NC/Clin	PPB study	4	4		0								
>Phase 1	Clin	PPB study	38	33		4								
Phase 1	Clin	FIM - HV	88	79	46	8	33	29	17	0				
Phase 2a	Clin	FIM - onco	100	92	54	13	33	29	17	0				
Phase 2a	Clin	FIP (start Ph-2)	96	96	67	13	38	38	21	4				
Phase 3-4	Clin	Patient studies	96	96	58	13	38	38	21	4				
phase 1 ->4	Clin	food effect, BA,...	100	88	67	13	38	38	21	4				
phase 1 ->4	Clin	DDI	100	96	71	13	38	38	21	4				
Phase 3-4	Clin	BE	100	83	54	13	38	38	21	4				



# Where do we struggle?

## 1.3 Scope

➤ This guideline describes the method validation that is expected for bioanalytical assays that are submitted to support regulatory submissions. The guideline is applicable to the validation of bioanalytical methods used to measure concentrations of chemical and biological drug(s) and their metabolite(s) in biological samples (e.g., blood, plasma, serum, other body fluids or tissues) obtained in pivotal nonclinical TK/PK studies that are used to make regulatory decisions and all phases of clinical trials in regulatory submissions. Full method validation is expected for the primary matrix(ces) intended to support regulatory submissions. Additional matrices should be partially validated as necessary. The analytes that should be measured in nonclinical and clinical studies and the types of studies necessary to support a regulatory submission are described in other ICH and regional regulatory documents.

- ‘pivotal’
- ‘support regulatory submissions’ vs. ‘make regulatory decisions’
- ‘primary matrix(ces)’ vs. ‘alternative matrices’
- ‘described in other ICH and regional regulatory documents’

# 'pivotal'

Suggest some wording in definitions on meaning of 'pivotal'

Questions: What is pivotal? The CRO may not know at the time and as previously discussed, the pivotal nature of a study may change over time and during the program.

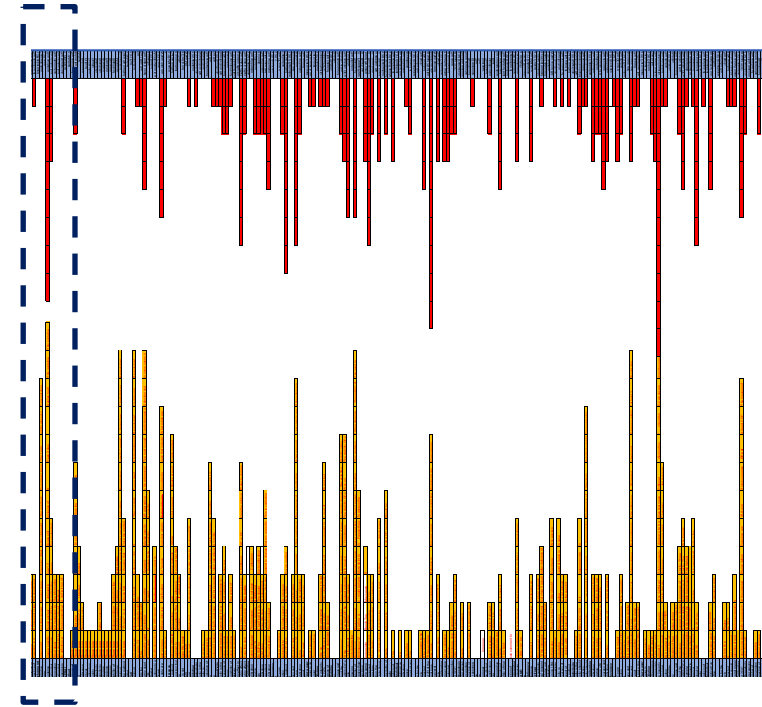
The term pivotal nonclinical TK/PK studies is ambiguous. Please include a listing of non-clinical studies for which "full" validation is required.

Pivotal non clinical studies: subject to interpretation ?

The results of pivotal nonclinical toxicokinetic (TK)/pharmacokinetic (PK) studies and of clinical trials ...  
Are all clinical studies pivotal??

Pivotal non clinical studies: subject to interpretation ?

What is a pivotal nonclinical TK study??



## Can 'pivotal' really be defined?

- Pivotal is also transient
  - Many “pivotal studies” will be superseded by another “pivotal study” in a next phase of development
- Pivotal for internal decision making vs. pivotal for regulatory decision making in a filling
- ...

# What defines a pivotal study?

- Pivotal:
  - /'pɪvətl/. - *adjective*
  - of crucial importance in relation to the development or success of something else.
- So not all can be pivotal.....But ask any project representative in drug R&D asking for budget of a study: *“is your crucial importance in relation to the development of our drug?”*
- And ask any bioanalytical expert the same question...

## ‘support regulatory submissions’ vs. ‘make regulatory decisions’

This guideline describes the method validation that is expected for **bioanalytical assays that are submitted to support regulatory submissions**. The guideline is applicable to the validation of bioanalytical methods used to measure concentrations of chemical and biological drug(s) and their metabolite(s) in biological samples (e.g., blood, plasma, serum, other body fluids or tissues) obtained in pivotal nonclinical TK/PK studies that are used **to make regulatory decisions** and all phases of clinical trials in regulatory submissions. Full method validation is expected for the primary matrix(ces) intended **to support regulatory submissions**.

Bioanalytical assays that are submitted to support regulatory submissions:

- *Relates to the assay - Implies no other assays can be submitted, even if scientifically appropriate*

To make regulatory decision

- *With a few exceptions (e.g. BE), impossible to know for the BA lab*

To support regulatory submissions

- *Semantics: supports vs. make ...It can be assumed, that, if in the file, everything “supports” a regulatory submission (either for decision making or as scientific supporting documentation).*

# 'primary matrix(ces)' vs. 'alternative matrices'

.....phases of clinical trials in regulatory submissions. Full method validation is expected for the primary matrix(ces) intended to support regulatory submissions. Additional matrices should be partially validated as necessary. The analytes that should.....

- Does 'partial' mean 'partial validation as per Guideline', or does it mean 'alternative approaches'?



= Manageable



In practice, this brings all analytes and matrices in scope

## ‘described in other ICH and regional regulatory documents’

.....The analytes that should be measured in nonclinical and clinical studies and the types of studies necessary to support a regulatory submission are described in other ICH and regional regulatory documents.

➤ An example for metabolites: ICH M3(R2)\*

ICH M3 (R2)

3. TOXICOKINETIC AND PHARMACOKINETIC STUDIES

\* [https://www.ich.org/fileadmin/Public\\_Web\\_Site/ICH\\_Products/Guidelines/Multidisciplinary/M3\\_R2/Step4/M3\\_R2\\_Guideline.pdf](https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M3_R2/Step4/M3_R2_Guideline.pdf)

## ICH M3 (R2)

### 3. TOXICOKINETIC AND PHARMACOKINETIC STUDIES

*In vitro* metabolic and plasma protein binding data for animals and humans and systemic exposure data (ICH S3A, Ref. 7) in the species used for repeated-dose toxicity studies generally should be evaluated before initiating human clinical trials. Further information on pharmacokinetics (PK) (e.g., absorption, distribution, metabolism and excretion), in test species and *in vitro* biochemical information relevant to potential drug interactions should be available before exposing large numbers of human subjects or treating for long duration (generally before Phase III). These data can be used to compare human and animal metabolites and for determining if any additional testing is warranted.

Nonclinical characterization of a human metabolite(s) is only warranted when that metabolite(s) is observed at exposures greater than 10% of total drug-related exposure and at significantly greater levels in humans than the maximum exposure seen in the toxicity studies. Such studies should be conducted to support Phase III clinical trials. For drugs for which the daily administered dose is <10 mg, greater fractions of the drug related material might be more appropriate triggers for testing. Some metabolites are not of toxicological concern (e.g., most glutathione conjugates) and do not warrant testing. The nonclinical characterization of metabolites with an identified cause for concern (e.g., a unique human metabolite) should be considered on a case-by-case basis.



Pretty clear....

So why do we think the intended (and even desired) scope for metabolites is this broad?

**INTENDED SCOPE**

	study	additional info on study
pre-phase 1	NC	dose range study in rat
pre-phase 1	NC	rat/dog/... PK
pre-phase 1	NC	28d GLP
Phase 1	NC	6m GLP
>phase 1	NC	mechanistic PK/TK
all phases	NC/Clin	PPB study
>Phase 1	Clin	PPB study
Phase 1	Clin	FIM - HV
Phase 2a	Clin	FIM - onco
Phase 2a	Clin	FIP (start Ph-2)
Phase 3-4	Clin	Patient studies
phase 1 ->4	Clin	food effect, BA,...
phase 1 ->4	Clin	DDI
Phase 3-4	Clin	BE

		plasma is the primary matrix			
mat		plasma	plasma	plasma	plasma
	dosed drug	M-active*	M > 10%*	M - not active	
Anal		0	0	0	
		13	13	0	
		96	88	13	
		100	100	17	
		21	21	4	
		4	4	0	
		38	33	4	
		88	79	46	8
		100	92	54	13
		96	96	67	13
		96	96	58	13
		100	88	67	13
		100	96	71	13
		100	83	54	13

33	
79	46
92	54
96	67
96	58
88	67
96	71
83	54

**DESIRED SCOPE**

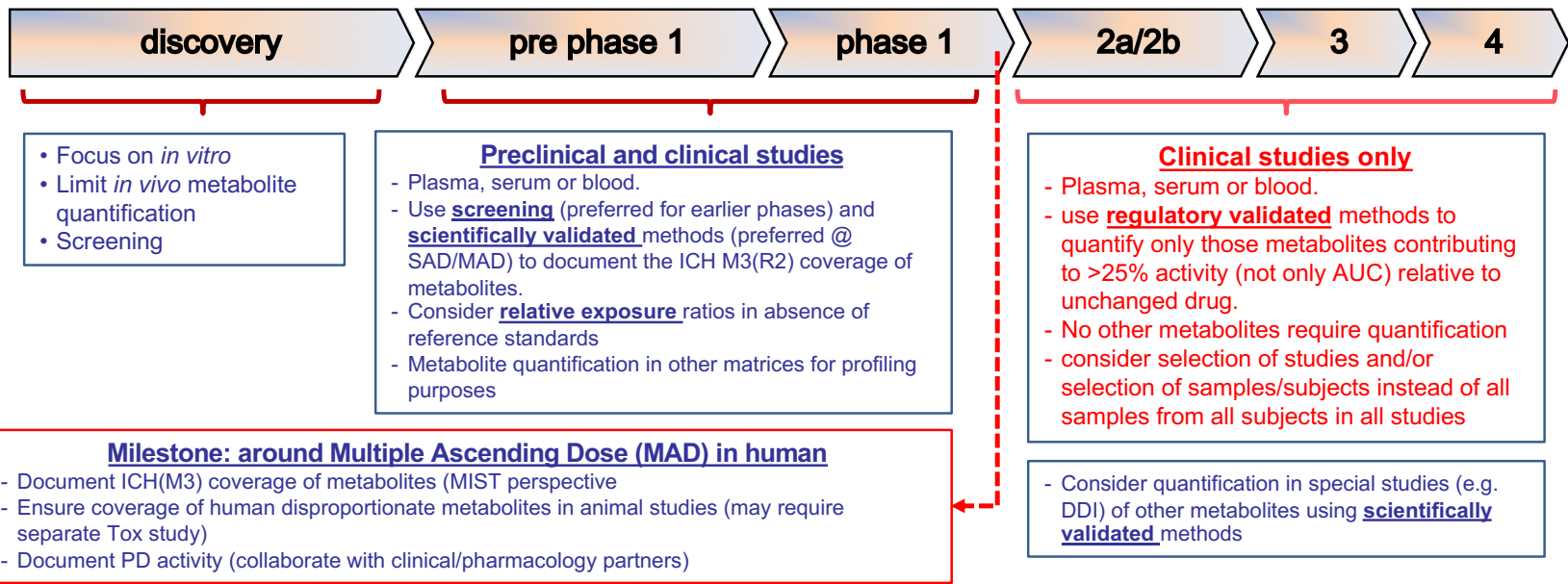
	study	additional info on study
pre-phase 1	NC	dose range study in rat
pre-phase 1	NC	rat/dog/... PK
pre-phase 1	NC	28d GLP
Phase 1	NC	6m GLP
>phase 1	NC	mechanistic PK/TK
all phases	NC/Clin	PPB study
>Phase 1	Clin	PPB study
Phase 1	Clin	FIM - HV
Phase 2a	Clin	FIM - onco
Phase 2a	Clin	FIP (start Ph-2)
Phase 3-4	Clin	Patient studies
phase 1 ->4	Clin	food effect, BA,...
phase 1 ->4	Clin	DDI
Phase 3-4	Clin	BE

		plasma is the primary matrix			
mat		plasma	plasma	plasma	plasma
	dosed drug	M-active*	M > 10%*	M - not active	
Anal		0	0	0	
		4	4	0	
		74	52	0	
		91	87	9	
		17	17	4	
		0	0	0	
		22	17	0	
		57	43	17	0
		78	61	26	4
		96	87	61	9
		96	83	48	9
		87	65	43	4
		100	91	61	9
		100	70	52	4

17	
43	17
61	26
87	61
83	48
65	43
91	61
70	52

# The 2016 EBF Recommendation – *in vivo* quantification \*

When using AMS or <sup>14</sup>C, use scientific criteria relevant to the technology.  
Be cautious not to mix up profiling and quantification purposes



\* Best practices for metabolite quantification in drug development: updated recommendation from the European Bioanalysis Forum *Bioanalysis*, 2016, 8(12), 1297-1305

# Suggested changes to Background and Scope - 1

## 1.2 Background

Concentration measurements of chemical and biological drug(s) and their metabolite(s) in biological matrices are an important aspect of drug development. The results of pivotal nonclinical toxicokinetic (TK)/pharmacokinetic (PK) studies and of clinical trials, including comparative bioavailability/ bioequivalence (BA/BE) studies, are used to make regulatory decisions regarding the safety and efficacy of drug products. It is therefore critical that the bioanalytical methods used are well characterised, appropriately validated and documented in order to ensure reliable data to support regulatory decisions.

Concentration measurements of chemical and biological drug(s) and their metabolite(s) in biological matrices are an important aspect of drug development. *The results of studies employing such methods contribute to regulatory decisions regarding the safety and efficacy of drug products.* It is therefore critical that the bioanalytical methods used are well characterised, appropriately validated and documented in order to ensure reliable data to support regulatory decisions.

*(proposal deconvolutes background from scope)*

# Suggested changes to Background and Scope - 2

## 1.3 Scope

This guideline describes the method validation that is expected for bioanalytical assays that are submitted to support regulatory submissions. The guideline is applicable to the validation of bioanalytical methods used to measure concentrations of chemical and biological drug(s) and their metabolite(s) in biological samples (e.g., blood, plasma, serum, other body fluids or tissues) obtained in pivotal nonclinical TK/PK studies that are used to make regulatory decisions and all phases of clinical trials in regulatory submissions. Full method validation is expected for the primary matrix(ces) intended to support regulatory submissions. Additional matrices should be partially validated as necessary. The analytes that should be measured in nonclinical and clinical studies and the types of studies necessary to support a regulatory submission are described in other ICH and regional regulatory documents.

This guideline describes the method validation that is expected for bioanalytical assays that are submitted to support regulatory submissions. The guideline is applicable to the validation of bioanalytical methods used to measure concentrations of chemical and biological drug(s) and their metabolite(s) in biological samples (e.g., blood, plasma, serum, other body fluids ~~or tissues~~) obtained **in nonclinical TK studies falling under the scope of the GLPs that are used to make regulatory decisions, nonclinical PK studies that are conducted as surrogates for clinical studies, and all phases of clinical trials in regulatory submissions for which a primary objective of the study is to assess, compare or characterize drug exposure.** Full method validation is expected for the primary matrix(ces) intended to support regulatory submissions. **Primary matrix(ces) are identified based on the objective(s) of individual studies and these should be indicated in the study protocol or sample analysis plan. For non-primary analytes/matrices validation should be performed in line with the anticipated use of the data, using the appropriate/applicable principles (i.e. partial validation or alternative approaches).** The analytes that should be measured in nonclinical and clinical studies and the types of studies necessary to support a regulatory submission are described in other ~~ICH and regional~~ regulatory documents. *(propose to delete the last sentence as other regulatory documents hardly describe this)*

## Suggested changes to Background and Scope - 3

### 1.3 Scope - cntd

For studies that are not submitted for regulatory approval or not considered for regulatory decisions regarding safety, efficacy or labelling (e.g., exploratory investigations), applicants may decide on the level of qualification that supports their own internal decision making.

For studies that are not submitted for regulatory approval ~~or not considered for regulatory decisions regarding safety, efficacy or labelling (e.g., exploratory investigations)~~, applicants may decide on the level of qualification that supports their own internal decision making.  
*(one does not know a priori if a study will be considered for regulatory decisions, thus in practice, only limiting to studies not included in submissions is possible)*

## 2.c. Some highlights from the industry FB

### A few Stability paragraphs

# Comments on Stability

## a picture tells and contains a 1000 words..

<p><b>3.2.8 Stability</b></p> <p>Stability of the analyte in the studied matrix is evaluated using low and high concentration stability QCs. Aliquots of the low and high stability QCs are analysed at time zero and after the applied storage conditions that are to be evaluated. A minimum of three stability QCs should be prepared and analysed per concentration level/storage condition/temperature.</p> <p>The stability QCs are analysed against a calibration curve, obtained from freshly spiked calibration standards in a run with its corresponding freshly prepared QCs or QCs for which stability has been proven. The mean concentration at each QC level should be within <math>\pm 15\%</math> 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 stability 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.</p> <p>If multiple analytes are present in the study samples (e.g., studies with a fixed combination, or due to a specific drug regimen) the stability test of an analyte in matrix should be conducted with the matrix containing all of the analytes.</p> <p>The following stability tests should be evaluated:</p> <ol style="list-style-type: none"> <li>Stability of stock and working solutions The stability of the stock and working solutions of the analyte and IS should be determined under the storage conditions used during the analysis of study samples by using the lowest and the highest concentrations of these solutions. They are assessed using the response of the detector. Stability of the stock and working solutions should be tested with an appropriate dilution, taking into consideration the linearity and measuring range of the detector. If the stability varies with concentration, then the stability of all concentrations of the stock and working solutions needs to be assessed. If no isotopic exchange occurs for the stable isotope-labelled IS under the same storage conditions as the analyte for which the stability is demonstrated, then no additional stability determinations for the IS are necessary. If the reference standard expires, or it is past the retest date, the stability of the stock solutions made previously with this lot of reference standard are defined by the expiration or retest date established for the stock solution. The routine practice of making stock and working solutions from reference standards solely for extending the expiry date for the use of the reference standard is not acceptable.</li> <li>Freeze-thaw matrix stability: To assess the impact of repeatedly removing samples from frozen storage, the stability of the analyte should be assessed after multiple cycles of freezing and thawing. Low and high stability QCs should be thawed and analysed according to the same procedures as the study samples. Stability QCs should be kept frozen for at least 12 hours between the thawing cycles. Stability QCs for freeze-thaw stability should be assessed using freshly prepared calibration standards and QCs or QCs for which stability has been proven. The number of freeze-thaw cycles validated should equal or exceed that of the freeze-thaw cycles undergone by the study samples, but a minimum of three cycles should be conducted.</li> <li>Bench top (short-term) matrix stability: Bench top matrix stability experiments should be designed and conducted to cover the laboratory handling conditions for the study samples. Low and high stability QCs should be thawed in the same manner as the study samples and kept on the bench top at the same temperature and for at least the same duration as the study samples. The total time on the bench top should be concurrent; it is not acceptable to use additive exposure to bench top conditions (i.e., adding up time from each freeze-thaw evaluation is not acceptable). Low and high stability QCs should be thawed in the same manner as the study samples and kept on the bench.</li> <li>Processed sample stability: The stability of processed samples, including the time until completion of analysis (in the autosampler/instrument), should be determined. For example (i) Stability of the processed sample at the storage conditions to be used during the analysis of study samples (dry extract or in the injection phase) (ii) On-instrument/ autosampler stability of the processed sample at injector or autosampler temperature.</li> <li>Long-term matrix stability: The long-term stability of the analyte in matrix stored in the freezer should be established. Low and high stability QCs should be stored in the freezer under the same storage conditions and at least for the same duration as the study samples. For chemical drugs, it is considered acceptable to extrapolate the stability at one temperature (e.g., <math>-20^{\circ}\text{C}</math>) to lower temperatures (e.g., <math>-70^{\circ}\text{C}</math>). For biological drugs, it is acceptable to apply a bracketing approach, e.g., in the case that the stability has been demonstrated at <math>-70^{\circ}\text{C}</math> and at <math>-20^{\circ}\text{C}</math>, then it is not necessary to investigate the stability at temperatures in between those two points at which study samples will be stored.</li> </ol> <p>In addition, the following test should be performed if applicable:</p> <ol style="list-style-type: none"> <li>Whole blood stability: 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 or serum, 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.</li> </ol>
--

<p>Stability of the analyte in the studied matrix is evaluated using low and high concentration stability QCs. Aliquots of the low and high stability QCs are analysed <del>at time zero and</del> after the applied storage conditions that are to be evaluated. A minimum of three aliquots of each of the stability QCs should be stored and analysed per concentration level/storage condition/temperature. <del>Analysis of the stability QCs prior to storage (e.g. at t=0) may be informative with respect to confirming that they have been correctly prepared, but is not required. (this proposal is in alignment with Health Canada expectations. No clear if EPPIA wants to push back)</del></p> <p>The stability QCs are analysed against a calibration curve, obtained from freshly spiked calibration standards in a run with its corresponding freshly prepared QCs or QCs for which stability has been proven. The mean concentration at each QC level should be within <math>\pm 15\%</math> of the nominal concentration. <del>If the concentrations of the study samples are consistently higher than the ULOQ of the calibration range, Stability should be demonstrated at a concentration within 25% of the maximum observed in study samples. Hence, additional stability assessment is necessary in the event that study sample concentrations greater than 125% of that of the high stability QC are measured in study samples, unless it is recognised and documented that this is not <del>may not be possible in nonclinical studies</del> due to solubility limitations.</del> (25% specification based on the fact that High QC is approximately within 25% of the ULOQ of the standard curve)</p> <p>If multiple analytes are present in the study samples (e.g., studies with a fixed combination, or due to a specific drug regimen) the stability test of an analyte in matrix containing all dosed compounds should be considered. In the case of a fixed combination stability information of the combination dosage form may be considered. <del>(would delete this sentence as it is scientifically not relevant and in many cases difficult to obtain) In the case of a drug regimen, the known chemistry and stabilities of the individually dosed drugs should be used as a basis for determining whether additional stability studies are needed. DDI studies are not in scope of this requirement</del></p> <ol style="list-style-type: none"> <li>Stability of stock and working solutions The stability of the stock and working solutions of the analyte and IS should be determined under the storage conditions used during the analysis of study samples by using the lowest and the highest concentrations of these solutions. <del>They are assessed using the response of the detector. Stability of the stock and working solutions should be tested with an appropriate dilution, taking into consideration the linearity and measuring range of the detector. If the stability varies with concentration, then the stability of all concentrations of the stock and working solutions needs to be assessed. If no isotopic exchange occurs for the stable isotope-labelled IS under the same storage conditions as the analyte for which the stability is demonstrated, then no additional stability determinations for the IS are necessary. If the reference standard expires, or it is past the retest date, the stability of the stock solutions made previously with this lot of reference standard are defined by the expiration or retest date established for the stock solution. The routine practice of making stock and working solutions from reference standards solely for extending the expiry date for the use of the reference standard is not acceptable. (reference to methodology removed to prevent flexibility)</del></li> <li>Freeze-thaw matrix stability: To assess the impact of repeatedly removing samples from frozen storage, the stability of the analyte should be assessed after multiple cycles of freezing and thawing. Low and high stability QCs should be thawed and analysed according to the same procedures as the study samples. Stability QCs should be kept frozen for at least 12 hours between the thawing cycles. Stability QCs for freeze-thaw stability should be assessed using freshly prepared calibration standards and QCs or QCs for which stability has been proven. The number of freeze-thaw cycles validated should equal or exceed that of the freeze-thaw cycles undergone by the study samples. <del>but a minimum of three cycles should be conducted.</del></li> </ol> <p>Low and high stability QCs should be thawed in the same manner as the study samples and kept on the bench top at the same temperature and for at least the same duration as the study samples. The total time on the bench top should be concurrent; it is not acceptable to use additive exposure to bench top conditions (i.e., adding up time from each freeze-thaw evaluation is not acceptable). <del>Low and high stability QCs should be thawed in the same manner as the study samples and kept on the bench</del></p> <ol style="list-style-type: none"> <li>Processed sample stability: The stability of processed samples, including the time until completion of analysis (in the autosampler/instrument), should be determined if quality control samples are not processed contemporaneously with study samples. The assessment is conducted by analyzing aged extracts of quality control samples against freshly prepared calibrators. Data generated during assay validation should support the longest period that is anticipated to elapse between sample extraction and analysis. <del>For example (i) Stability of the processed sample at the storage conditions to be used during the analysis of study samples (dry extract or in the injection phase) (ii) On-instrument/ autosampler stability of the processed sample at injector or autosampler temperature.</del></li> <li>Long-term matrix stability: The long-term stability of the analyte in matrix stored in the freezer should be established. Low and high stability QCs should be stored in the freezer under the same storage conditions and at least for the same duration as the study samples. <del>For chemical drugs, it is considered acceptable to extrapolate the stability at one temperature (e.g., <math>-20^{\circ}\text{C}</math>) to lower temperatures (e.g., <math>-70^{\circ}\text{C}</math>). For biological drugs, it is acceptable to apply a bracketing approach, e.g., in the case that the stability has been demonstrated at <math>-70^{\circ}\text{C}</math> and at <math>-20^{\circ}\text{C}</math>, then it is not necessary to investigate the stability at temperatures in between those two points at which study samples will be stored.</del></li> </ol> <p>In addition, the following test should be performed if applicable:</p> <ol style="list-style-type: none"> <li><del>Whole blood stability: Sample collection integrity: Sufficient attention should be paid to the stability integrity 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 sample at the time of sample collection. Conditions for sample collection should be identified during method development or validation. If the matrix used is plasma or serum, the stability of the analyte in blood should be evaluated considered during method development (e.g., using an exploratory method in blood), and, in the case of molecules that are, based on their structure, potentially unstable, assessed during method validation. The results of such assessments or, in the event they are not conducted, rationale for their absence, should be provided in the Validation Report.</del></li> </ol>
---

**Let's pick 2...**



## FDC

To date, industry has no scientific data to support a claim that one drug has an impact on the stability of another drug in a biological matrix. And the experiment was performed hundreds of times...

If multiple analytes are present in the study samples (e.g., studies with a fixed combination, or due to a specific drug regimen) the stability test of an analyte in matrix should be conducted with the matrix containing all of the analytes.

If multiple analytes are present in the study samples (e.g., studies with a fixed combination, or due to a specific drug regimen) **the stability test of an analyte in matrix containing all dosed compounds should be considered.** In the case of a fixed combination stability information of the combination dosage form may be considered. *(would delete this sentence as it is scientifically not relevant and in many cases difficult to obtain)* In the case of a drug regimen, the known chemistry and stabilities of the individually dosed drugs should be used as a basis for determining whether additional stability studies are needed. **DDI studies are not is scope of this requirement**

## e.g. LT -20/-70° C

To date, industry has no scientific data to support a claim that a protein is instable in an LTS experiment @ -70° when it was stable @ -20° . And the experiment was performed hundreds of times... 1F/T may impact

5) Long-term matrix stability: The long-term stability of the analyte in matrix stored in the freezer should be established. Low and high stability QCs should be stored in the freezer under the same storage conditions and at least for the same duration as the study samples. For chemical drugs, it is considered acceptable to extrapolate the stability at one temperature (e.g., -20°C) to lower temperatures (e.g., -70°C). For biological drugs, it is acceptable to apply a bracketing approach, e.g., in the case that the stability has been demonstrated at -70°C and at -20°C, then it is not necessary to investigate the stability at temperatures in between those two points at which study samples will be stored.

5) Long-term matrix stability: The long-term stability of the analyte in matrix stored in the freezer should be established. Low and high stability QCs should be stored in the freezer under the same storage conditions and at least for the same duration as the study samples. ~~For chemical drugs, it is considered acceptable to extrapolate the stability at one temperature (e.g., -20°C) to lower temperatures (e.g., -70°C). For biological drugs, it is acceptable to apply a bracketing approach, e.g., in the case that the stability has been demonstrated at -70°C and at -20°C, then it is not necessary to investigate the stability at temperatures in between those two points at which study samples will be stored.~~

## 2.c. Some highlights from the industry FB Method development requirements

- The risk of current method development requirements:
  - Loss of scientific freedom
  - Industry take documentation to a level which is unmanageable
  - HA start expecting
- The proposal
  - Paragraph 2.1: "Method Development" carries the risk of becoming overinterpreted and are increasing the resource requirements for industry, whilst stifling scientific freedom required in the method development arena (and not aligned with the mission of ICH).
  - For "Method Development," we suggest to limit to scope to changes to already validated methods in later stages of development.

## 2.c. Some highlights from the industry FB Documentation requirements

- “Table 1: Documentation” carries the risk of becoming overinterpreted and are increasing the resource requirements for industry, whilst stifling scientific freedom required (and not aligned with the mission of ICH).
- We suggest to limit the requirements in table 1 to BA/BE-studies, and allow reporting of other studies to be less detailed (i.e. less in reports but allow documentation to be available at the analytical site)

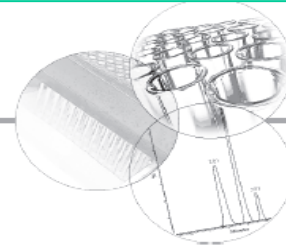
## 2.c. Some highlights from the industry FB Incurred Sample Reanalysis

# EBF position on ISR

## WHITE PAPER

*For reprint orders, please contact [reprints@future-science.com](mailto:reprints@future-science.com)*

**Incurred sample reproducibility:  
views and recommendations by the  
European Bioanalysis Forum**



White Paper

SPECIAL FOCUS ISSUE: Incurred sample reproducibility (ISR)

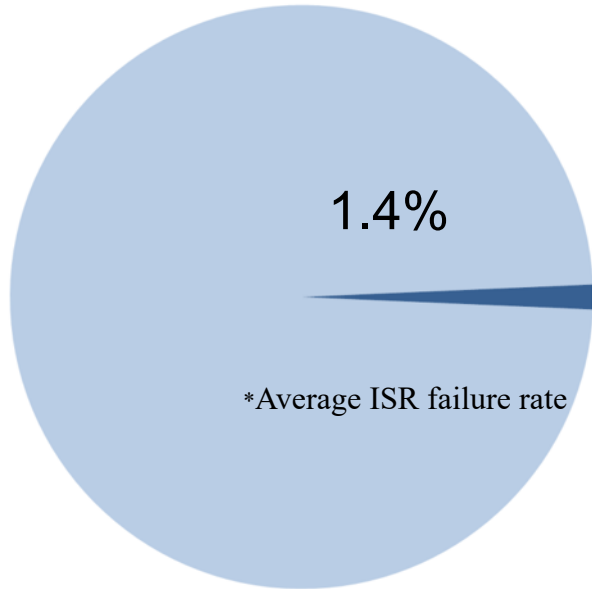
*For reprint orders, please contact: [reprints@future-science.com](mailto:reprints@future-science.com)*

**Incurred sample reproducibility: 10 years of  
experiences: views and recommendations  
from the European Bioanalysis Forum**

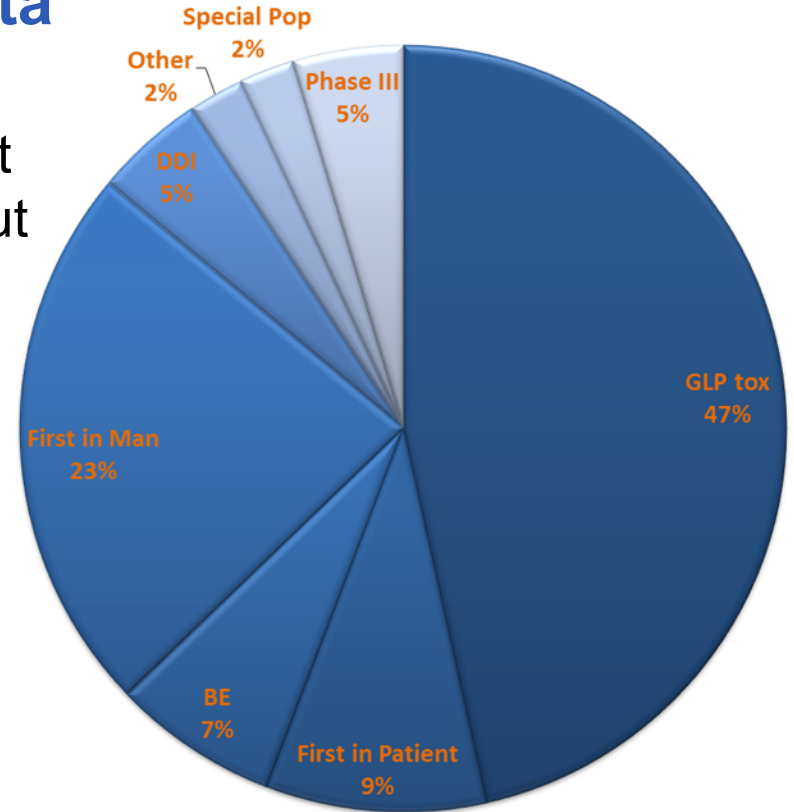
**Bioanalysis**



# Industry position based on data



1.4% = about 80 studies out of 5500



\*n > 5500 studies

## “Pre-ICH” EBF position on ISR

- ISR failure rate was, in a survey with more than 5500 studies including regulated Bioanalysis and ISR, low (app. 1.5%) and failures were mostly in earlier development studies.
  - Did we ever consider if Failed ISR has a real impact on patient safety?
- Based on current experiences (1.5% ISR failure rate), causes and impact of failed ISR, the 10 + 5 % repeats is a high number not adding value.
- The number of ISR should be aligned with number of spiked QC's in a run (5% - in alignment with AAPS)\*
- Consider Fixed number approach as an alternative to Fixed ratio?

# Feedback from EBF & EFPIA

There should be a cap on the number of ISR samples that are needed. For large studies, it is not statistically relevant and a waste of resources to allocate to performing such a large number of runs for ISR. We suggest adding a cap for the number of ISR samples needed.

Are Ph-III pivotal studies excluded from the list? A better definition of early would be helpful

add "GLP-regulated toxicokinetic" to clearly define when ISR needs to be done thereby avoiding ambiguity with respect to the scope.

"main nonclinical TK studies" is rather vague.

ISR will not allow to identify the described effects/differences between study sample and calibrators/QCs. If the study sample shows any of the described effects which is responsible for a bias with calibrators/QCs, reanalysis will give the identical (biased) result.

Please focus on the fact that ISR provides information of reproducibility of the assay using real samples.

The meaning of the term "main", used for nonclinical TK study, is not clear. The term "pivotal" is not clear; add the meaning of "PIVOTAL" in the glossary

Minimum number relevant? in case of limited sample volume, pooling may be unavoidable

30 samples should be sufficient power in any study size

ISR should be able to be conducted on the same day, especially if there are stability concerns.

"flyers" should not trigger reanalysis - really?

## In summary...EBF/EFPIA Position

Haven't we done enough to refine our process?

Please consider to provide a cap, i.e. a maximum for sample number to be analyzed as part of ISR. There are strong scientific data suggesting that reanalyses of large portions of samples do not added scientific value. Literature suggests that 30 samples should be sufficient power in any study size. A consensus proposal could be: For ISR, reanalyse 10% of the study of samples, with a minimum of 20 and a maximum of 100 samples.

## 2.c. Some highlights from the industry FB Partial & Cross validation

## Partial Validation

- Except for a few minor worries, industry has no real comments on partial validation
- Training may be needed to emphasise that Partial validation is only to document changes to an already fully validated method. After 30y, industry still is confused on this simple principle
- One Concern: Risk of transferring **stability data** between CRO/Pharms (what happens if the stability data is deficient, who gets the regulatory citing?). A company/CRO may consider stability data owned by another company as not shareable

# Cross validation however...

## 6.2 Cross Validation

Cross validation is required to compare data under the following situations:

- Data are obtained from different fully validated methods within a study
- Data are obtained from different fully validated methods across studies that are going to be combined or compared to support special dosing regimens, or regulatory decisions regarding safety, efficacy and labelling.
- Data are obtained within a study from different laboratories with the same bioanalytical method.

Cross validation is not generally required to compare data obtained across studies from different laboratories using the same validated method at each site.

Cross validation should be performed in advance of study samples being analysed, if possible.

Cross validation should be assessed by measuring the same set of QCs (low, medium and high) in triplicate and study samples that span the study sample concentration range (if available  $n \geq 30$ ) with both assays or in both laboratories.

Bias can be assessed by Bland-Altman plots or Deming regression. Other methods appropriate for assessing agreement between two assays (e.g., concordance correlation coefficient) may be used too. Alternatively, the concentration vs. time curves for incurred samples could be plotted for samples analysed by each method to assess bias. If disproportionate bias is observed between methods, the impact on the clinical data interpretation should be assessed.

The use of multiple bioanalytical methods in the conduct of one comparative BA/BE study is strongly discouraged.

## Cross validation

- A lot of comments were given asking for “criteria”
- From all comments, it looks like industry missed the new philosophy of the draft guideline?

*Bias can be assessed by Bland-Altman plots or Deming regression. ....If disproportionate bias is observed between methods, the impact on the clinical data interpretation should be assessed.*

- Does this mean that, if the run passes, a cross validation cannot fail?
- Is the intention to investigate and documents a potential bias across studies?

**If so, we are entering in new territory, at least for BA... (next slide)?**

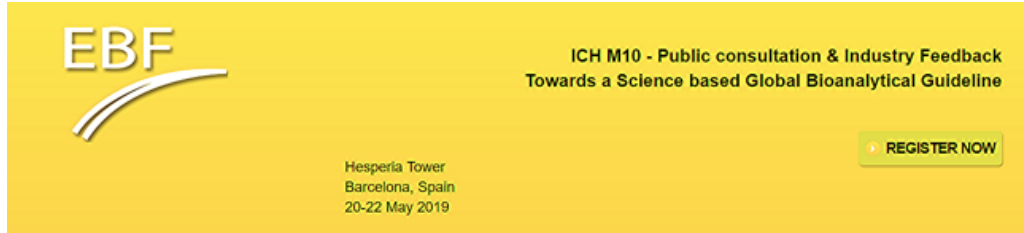


# Cross validation

Questions that arise include:

- What is “*a disproportionate bias*”? And who owns “*the impact on the clinical data interpretation should be assessed*” ?
- Who will decide on actions to be taken?
  - Will the BA scientist provide a new set of corrected concentrations?
  - Is it the PK scientist? The regulators?
  - And when, why , how....
- And above all, do we currently have the experience in industry to execute this new requirement?
  - **More context will be required** → survey revealed industry didn't recognise the expectation
  - **Intensive training will be required** to manage correct use (incl. documentation and responsibilities)

Intensive  
**TRAINING**  
&  
**COMMUNICATION**  
will be needed



- All details from the discussion from Barcelona can be found on the EBF website:
- All slides: <http://www.e-b-f.eu/fw201905-slides/>
- Conclusion slides: <http://www.e-b-f.eu/wp-content/uploads/2019/06/FW201905-061.-Recommendations-from-the-EBF-Spring-FW-2019.pdf>
- This were the basis of the comments that were submitted to the EWG via EMA (both from EBF and EFPIA)

## **If I am allowed personal note... I too wondered why we got so many industry comments on a “30-y old Guideline”**

1. Some paragraphs in ICH M10 are new and are not part of any Guideline. As such, they have not been discussed in full
2. Most comments are not related to “harmonisation” per se, but to continued challenge from industry on regional guidelines
3. Fear that global guideline has a wider scope than any current regional guideline

# Acknowledgements

- EBF community
- Delegates to EBF Autumn Focus Workshop (Malaga, September, 2019)
- Delegates to EBF/AAPS/JBF/CBF sister meeting (Barcelona, May, 2019)
- All of you

# Contact Information

Questions: [info@e-b-f.eu](mailto:info@e-b-f.eu)



European Bioanalysis Forum vzw,  
[www.e-b-f.eu](http://www.e-b-f.eu)