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WHITE PAPER

A European Bioanalysis Forum recommendation for requiring a context-of-use statement for successful development and validation of biomarker assays

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ABSTRACT

The European Bioanalysis Forum, alongside key industry stakeholders, has been driving the discussions around the implementation of context-of use for biomarker assays to ensure that these assays are validated appropriately depending on their purpose. Insights into understanding why the implementation of context-of-use in assay strategies has also shown that the key stakeholder, or requester for the biomarker data, is responsible for providing the context-of-use statement for all biomarker assay requests. Experts from across the industry haves repeatedly sought a cross-industry recommended format in which the context-of-use statement could be provided. In this manuscript, the European Bioanalysis Forum suggests a format for this.

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1. Background

Biomarkers are key to successful drug development. Given the high attrition rate in drug development, especially in clinical proof-of-concept studies, there is a strong need for improving our quantitative predictions, translational concepts and patient selection using an effective biomarker (BM) strategy. Bioanalytical scientists are responsible for implementing how each BM is measured appropriately. However, to achieve this successfully, many organizational and strategic aspects have been shown to be instrumental. Understanding the context-of-use (CoU) of a BM is essential for the validation of the BM assay, the reliability of the generated data and how the data will support the clinical development and allow informed decision-making [1,2,3,4,5,6]. CoU and is therefore integral to any BM strategy. Arguably, the most crucial tool to support a successful BM strategy is the CoU statement for each BM.

There are plenty of examples that illustrate the value of CoU strategies, or the risk of failing drug development when setting up BM assays without a defined CoU, as discussed at a European Bioanalysis Forum (EBF) Focus Workshop [5]. Having a global, cross-industry understanding of CoU, its importance in BM-guided drug development, how to implement it in BM strategies and who needs to be involved in implementing it. It is not just the scientific and analytical challenges that need to be overcome, but also, often forgotten and one of the key recommendations from the EBF, strategic challenges such as communication, stakeholder management and operational issues [1,3,6], which have resulted in over a decade of debate and discussion. This set of CoU principles was first discussed in 2012 by the EBF in a recommendation paper on method establishments and bioanalysis of BMs in support of drug development [1] and described the need for scientific rationale to drive the implementation of specific bioanalysis strategies for BMs, albeit the CoU vocabulary was not explicitly used at that time. That EBF recommendation paper described four pillars that support a decision tree for implementation of a bioanalytical strategy for BMs. These four pillars included: understanding

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the biology; knowing the scientific and regulatory needs driven by the phase of development that the molecule is in; understanding the decisions that a project team would make based on the BM assay results; and the potential influence, or more specifically, lack of guidance at that time. Over time, a considerable portion of the bioanalytical community has misapplied the bioanalytical method validation (BMV) guideline from the FDA from 2018 [7], implementing the recommendations for pharmacokinetics (PK) assays which is a practice that the EBF does not endorse for BM assays. This guideline is the only one with a section on BMs. Most importantly however, in the BM section of the guidance is this quote:

> *The approach used for drug assays should be the starting point for validation of biomarker assays, although the FDA realizes that some characteristics may not apply or that different considerations may need to be addressed.*

The EBF rapidly recognized a fifth and equally critical pillar: communication. Timely and frequent communication on CoU and assay requirements between all stakeholders is important in a cross-functional setting to understand the biology of the target analyte, and to understand how the data will be used by drug development teams over time, so that specific analytical requirements are discussed and understood before any bioanalytical strategy is put in place. Although discussed at meetings since 2012, this fifth pillar was first published in 2018 [8].

In a recommendation paper from 2020 [3] as an update to the EBF recommendation paper on BM assays from 2012, the EBF first linked the CoU vocabulary. The EBF recommendations here included that the CoU must first be defined and agreed upon and understood by all stakeholders and EBF recommends this to fully understand what questions the BM data will address. In other words, every assay request begins with the question why, and a series of questions are suggested to understand the bottom line: what is the scientific rationale to measure this BM, in other words, the purpose in fit-for-purpose? This should then result in a fully defined, documented definition of the purpose, in other words, the CoU of the BM in question. The CoU statement can then serve to identify the fit-for-purpose bioanalytical strategy, for example the type of assay required, measuring free or total BM levels, development of an in-house assay or use(/repurpose) of a commercial (diagnostic) kit, single analyte or multiplex, research use or diagnostic, etc. Then the format of the assay, critical reagents, technology choice(s) with pros and cons and appropriate BM sample selection to characterize the assay can be chosen to develop and evaluate the method, followed by the appropriate assay acceptance criteria. Again, the key here would be to avoid altogether the implementation of any PK standard operating procedure (SOP) developed to support bioanalysis for BMs, in other words, the misapplication of the BMV guidelines [7] to BM assays.

In absence of a common ground for the industry and regulatory authorities, the EBF BM teams continued to address the overarching question: what is slowing down the implementation of CoU principles for BMs across the industry? The EBF identified gaps within the bioanalytical community around having a common understanding and alignment of what CoU is, how to get the CoU information right, how CoU drives what is done in the lab and the importance of stakeholder engagement. With this, it was clear that the bioanalytical community needed to maintain the momentum of the ongoing discussion for clarity and alignment across the industry, let alone with stakeholders. The EBF then provided a subsequent recommendation paper on how this could work from an organizational design perspective both for sponsors as well as CROs [6].

Regardless of whether the BM activities are internal or externalized to a CRO, the EBF recommends that each key stakeholder that requests the BM data (the requester) delivers a documented, scientifically sound CoU statement for each BM to be measured. Only then the assay chosen can be validated for its defined purpose. Only when the CoU is clear can the data be fit-for-purpose. A recent paper describes the debate [9] and the authors' approach for implementing CoU principles. Herein the authors describe the need for the CoU and an understanding of the biology, plus knowledge of the study hypothesis and the planned data analysis of the BM of interest prior to any bioanalytical strategy and assay requirements. The EBF would add to this that it is essential and the responsibility of the stakeholder who requested the BM data, the requester being a team lead of a project, whether it is a clinician, a clinical pharmacologist, a BM team lead, a molecule team program lead, or other, to first provide a well-defined BM strategy, with prioritized BMs and to provide the CoU for each of these BMs to the bioanalytical expert. In every case, this should continue to be an iterative and bi-directional communication and discussion with the bioanalytical lead until all stakeholders understand and subscribe to what needs to be performed in the lab, as suggested in the 2012 EBF recommendation paper [1].

Thus, the BM team must first define the intended CoU for BM assays. The BM CoU statement can often be limited to a few sentences but detailed enough to define the purpose of the assay for each analyte. This statement needs to be understood and agreed upon by all stakeholders and documented in method summaries, validation plans,

and/or validation reports. Even in case when the BM is unknown, and a screening for BMs or a feasibility study is initiated to further understand the presence or value of a BM, if any, the CoU statement must be captured. Then it's possible to consider what makes sense technically from a bioanalytical perspective which then can lead to the appropriate assay characterization and application of an acceptance criteria. The impact in the end is: to ensure the appropriate interpretation of data for the best drug development strategy ultimately to serve patients.

The EBF created, in response to the discussions within the bioanalytical community, "roadshows" or "connect locally workshops", which highly encouraged the presence of stakeholders such as clinicians, clinical pharmacology representatives, BM leads and others to come together to learn about and further discuss the CoU principles. These have been relatively small, half-day events that have sought to broaden the understanding of the CoU principles and to bring ownership to requesters and therefore team leads across the industry. A very simplified description of CoU can be found on the FDA website [10], which supports a simpler CoU approach specific to BM qualification, as defined in the (Biomarkers, EndpointS and other Tools or BEST) Resource [11].

Going forward, and considering the input from all discussions mentioned above, the EBF feels that any CoU statement should at least contain the following information: elements that include BM identity, if available already, BM category, BM use, known endogenous BM level and variability, expected difference to be captured in what matrix and impact, all which should be documented as a statement wherever applicable and traceable, and updated over time for each assay as the CoU evolves. Of course, the field of biomarkers, biomarker measuring technologies and the intended uses of biomarker data within drug discovery and development is very broad. Thus, it would become difficult to define a template that is generally applicable for all cases. Our main focus herein is on soluble biomarker in biological fluids, but the same general categories apply for other CoU statements as well.

At minimum, a CoU statement should contain:

- BM identity: Name of the biomarker. This may include the uniprot number, a certain isoform, or the ability to distinguish free from drug bound fractions.
- BM category: This refers to the list of BEST [11] or additional categories (for example, target engagement) that describe the main purpose of the biomarker in the context of a study.
- BM use/purpose: The scientific and/or strategic rationale for measuring the biomarker, and how the data will be evaluated.
- BM biological context: What are the endogenous levels and how variable is the biomarker within a subject (for example, circadian rhythm) or between subjects of a population. In a more comprehensive CoU description this also may include details about the biological function of the biomarker and possible interactions with other molecules. These information helps to select the optimal analytical method and to define the critical analytical parameter that should be tested during method validation.
- BM change or treatment effect: What are the expected (concentration) changes during treatment or difference in levels between populations? What is the reference range applied (for example, for safety or diagnostic biomarker) or the cut-off level used for a certain decision (for example, for patient selection)?
- BM impact: What is the impact of the biomarker data on any decision? Are the data involved in any decision trees? This would allow a risk assessment considering business risk, regulatory risk and patient risk.

The information can be provided in a format suitable for communication with the bioanalytical scientist, for example, in a table format, or can be a comprehensive sentence/paragraph, which could look like this, for example, for the quantitation of a soluble biomarker:

> [A] is a [exploratory/potential valid/known valid) BM for [(i. disease/safety), (ii. response (PD)), (iii. patient selection), etc]. Baseline levels are expected to be at [concentration B] in [matrix C] from [patient population(s)/animal species(s) D]. The intra- and inter-subject variability is [known/unknown]. The hypothesis is, that BM [A] will [increase/decrease] after treatment over [time E] to a [known/unknown] extent. The impact of the data is to support decision [F].

Since there are so many potential CoU statements, we provide here a few examples of how a CoU statements could be delivered, inspired by a few BM categories of Biomarkers from BEST (Figure 1) [11].

In a next step, this CoU statement should then be translated by the bioanalytical team to help define the assay characterization and acceptance criteria. Again, this can take the format of a simple table all the way to a bioanalytical protocol, depending on how the laboratory or the communication lines between the lab and the requester are established, for example, in-house or in collaboration with an external laboratory. It is important that the proposed assay is discussed with the requester to ensure the assay will generate the desired outcome. In the fol-

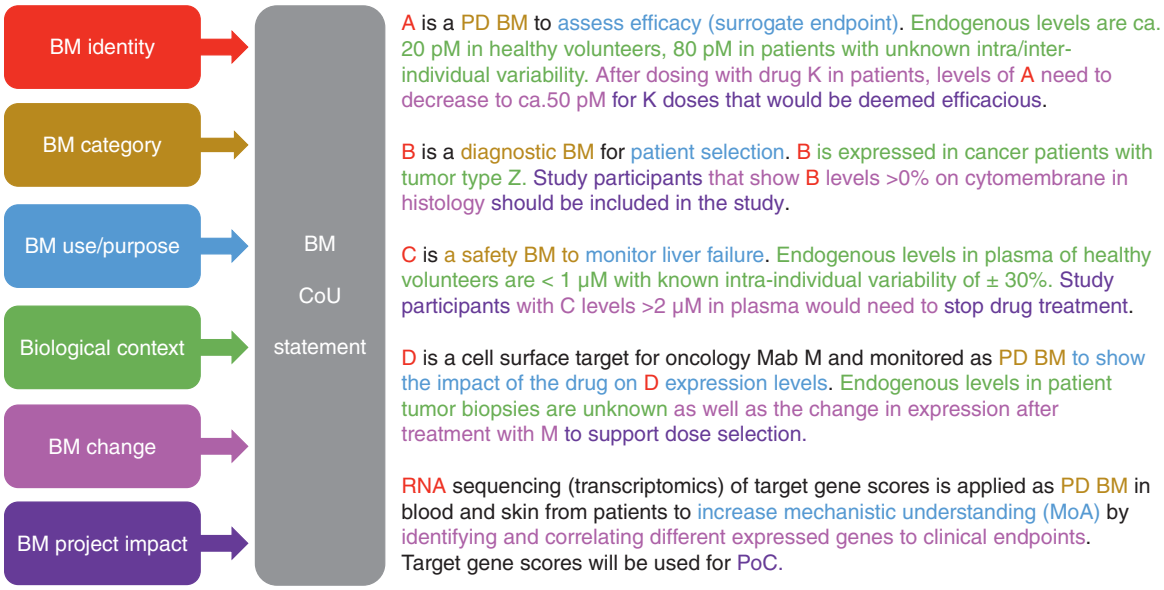

Figure 1. Five different examples in a visualized format of the CoU statement, with the BM identity as defined, BEST [11]-defined category of BM (yellow), the use of the BM (blue), the levels and variability of the BM (green), the potential changes in the BM (pink) and the impact of the BM data or decisions that could be made (purple). Inspiration for these figures in [12]. This information framework for the definition of CoU of a biomarker in a clinical study could be extended to the majority of biomarker uses independent from the measuring technology, including tests for panels of biomarkers or screening approaches, as shown in the RNA sequencing CoU statement example. Not every statement will include all elements, however this illustrates how CoU statements could look like.

lowing, we present two case studies in which the CoU is comprehensively described in a table format covering all six bullet points mentioned above, and a seventh one that addresses important logistical information about the planned study. The CoU content is translated into the bioanalytical strategy. The latter allows selection of the optimal assay and the definition of the required validation experiments and method acceptance criteria that would be necessary to confirm that the selected assay is fit for the pre-defined CoU.

In the above case study, the validation plan contained validation experiments that were performed over 15 runs on about 8 working days.

The validation plan of the above case study scheduled assay validation covered five runs (plates) over three working days. Thus, much shorter than the first case study. On the first view both case studies would look similar, but the different CoU statements were translated into two completely different validation packages. This is what fit-for-purpose assay validation means, a more advanced assay validation for a higher risk and impactful biomarker and a reduced core validation package for a low-risk biomarker. These considerations included:

- A more precise method in case of an expected small change of the biomarker after treatment,
- A more sensitive method to determine not only higher patient but also lower healthy volunteer level,
- A quasi-quantitative assay without proven parallelism if just change from baseline trends should be detected,
- A robust assay using monitoring samples and a bridging approach for lot-to-lot changes for long-term use of the method with comparable results,
- More interference tests in case of known interactions between analyte and other binding partners that may be present in the sample.

For primary end points, the EBF also recommends, that once this CoU statement per analyte is documented and the assay parameters evaluated and acceptance criteria defined, the teams can then seek interactions with health authorities to exchange on whether the BM bioanalytical strategy is scientifically sound. At this point for example, BM validation reports can then be submitted to health authorities if the data is supporting a primary end point (but not necessarily for secondary and/or exploratory end points in (non-)clinical protocols). If the validation reports are only describing secondary and/or exploratory end points, the EBF suggests that these reports do not need to be submitted, unless requested by health authorities. The EBF recommends having these upfront discussions with the health authorities prior to submission, to ensure alignment on this strategy.

Case 1	CoU	Information necessary for:	Translation into bioanalytical strategy
BM identity	Total soluble target XY (uniprot #)	Choice of specific assay	Ligand binding assay specific for total XY, no or little interference of drug only up to anticipated C_{max} drug concentration in highest dose cohort
BM category	Exploratory end point for indirect target engagement PD BM		
BM Use/purpose	Verification of proof of target engagement and pharmaceutical principle (together with free soluble target) and support of dose selection together with PK and safety data. XY concentration values will be used to verify a PK/PD model	Choice of assay, relative quantitative assay (=required parallelism) or quasi-quantitative assay	Proven parallelism in several individual matrix samples over a dilution range of two ten-fold dilutions at least. Revalidation with post-treatment study samples to cover high endogenous XY level. Definition of molecular weight for calibration standard to calculate molar concentrations
Biological context	XY is the soluble target of the antagonistic therapeutic anti-XY antibody Z. XY interacts with V in the circulation. The patient baseline levels are in the range of 2–20 ng/ml and about twofold higher than healthy volunteer level. The longitudinal biological variance is not known	Range of assay, minimum sensitivity, specificity, possible interference of interacting proteins	Test for interference of V. Lower limit of quantification should be in the low range of healthy volunteers at least. Estimation of the longitudinal biological variance of XY in healthy volunteers (biobanked sample sets of several donors)
BM change	XY may increase several fold after treatment due to half-life prolongation by drug-target complex formation. A possible feedback mechanism is unknown	Range of assay, precision, selection of QCs	Broad range of 2–3 orders of magnitude due to high range of expected XY concentrations (electrochemiluminescence immunoassay). Endogenous matrix QC + spiked matrix to cover upper range. Precision does not need to be very high (20–30% CV between-runs)
BM impact and risk assessment	Part of data package that would trigger continuation of drug development. Support of decision on future dose in further studies (together with free target, PK and safety data)	Risk assessment extent of assay validation	Patient risk is low (no direct influence on patient treatment or health), regulatory risk and business risk are moderate (influence on further clinical development) Comprehensive standard LBA assay validation with focus on robustness, sampling stability, parallelism and specificity
Data comparability and logistical study details	BM levels should be compared not only within this 4-year lasting study but also in further Phase II studies. Frequent interim data evaluations are planned after each dose cohort	Long term strategy and planning of materials and resources. Assay monitoring/QC charts Assay robustness to guarantee comparability of results over long term	Banking of reference standard, monitoring of assay performance by sample controls/sufficient QCs, bridging approach for QC lots and critical reagents, revalidation from time to time to confirm healthy volunteer population range and parallelism. Prospective isochronic long term stability up to 5 years. Blood sampling stability and robustness

2. CoU beyond BM assays

Several sponsors and contract research organizations (CROs) are moving away from the wrong practice of referencing PK SOPs for BM assays, and several such case studies have been presented at international forums. From the EBF Focus Workshop in 2022, and the EBF Open Symposium in 2023, there is evidence of the need for the implementation and broadening of the value of CoU principles, and that these principles are seen as critical [5,13].

Also, for immunogenicity assays, as the CoU statement can be impacted by the stage of development (nonclinical, clinical, Phase I vs. Phase III) and the immunogenicity risk assessment (low to medium *vs.* high risk). A well-defined CoU can drive which tier(s) of immunogenicity assessment are deployed, and therefore which assay(s) are appropriate. In turn, the CoU requires understanding the utility and limitation of the immunogenicity assay(s), and therefore the purpose of the assay and the decisions being made with the data can impact the assay characteristics needed and corresponding acceptance criteria. Even if an immunogenicity assay follows current regulatory guidance, the science may be flawed and this will not guarantee a successful submission [14]. This is especially important considering new modalities in drug discovery and development pipelines today.

In continuation, the EBF believes that the CoU principles can be applied for other types of assays and measurement technologies, such as qPCR [15]. There are many different CoUs for the various qPCR applications, and each CoU has its own performance requirements for the qPCR method. There is a desire for the harmonization of bioanalytical qPCR approaches, however most importantly: existing regulatory BMV guidance/guidelines written for PK assays using chromatographic and ligand bind-

Case 2	CoU	Information necessary for:	Translation into bioanalytical strategy
BM identity	BM AB (uniprot #), total isoforms that contain the cell binding domain (see literature)	Choice of specific assay	Commercial ELISA kit that has been mentioned in referenced literature
BM category	Exploratory end point for a physiological response PD BM		
BM Use/purpose	Hypothesis testing whether BM XY could be a used as physiological response biomarker in the indication diabetic nephropathy. Evaluation of BM results as average fold change from baseline treated vs. placebo cohorts	Choice of assay, relative quantitative assay (=required parallelism) or quasi-quantitative assay	Quasi-quantitative ELISA sufficient, in case of non-parallelism measurement of all samples in a fixed dilution and replacement of calibration curve by linear interpolation of normalized response values
Biological context	Urinary BM that originates from local, intrarenal production. Patient levels are about three-fold higher than healthy volunteer level (literature). Biological longitudinal variance in urine unknown	Range of assay, minimum sensitivity, specificity, possible interference of interacting proteins	Specificity information taken from vendor manual, no further experiments Lower limit of quantification should be in the low range of healthy volunteers at least Estimation of the longitudinal biological variance of XY possible from placebo patients, no additional experiment
BM change	XY may decrease after treatment to an unknown extent but maximal down to the healthy volunteer level	Range of assay, precision, selection of QCs	Limited range of 1–2 orders of magnitude sufficient due to limited biological inter-subject variability and low treatment effect. Two urine endogenous matrix QC (low and high) sufficient. No acceptance criterion on assay precision
BM impact and risk assessment	Supportive scientific data not solely used for any decisions	Risk assessment extent of assay validation	No patient risk, regulatory risk and business risk are very low (no influence on further clinical development) Basic LBA assay validation with focus on precision and stability
Data comparability and logistical study details	Comparability of results within this study only. Duration of study one year. Treatment period per patient 3 months. No interim data evaluation planned	Long term strategy and planning of materials and resources. Assay monitoring/QC charts Assay robustness to guarantee comparability of results over long term	Measurement of all samples of a patient together in the same run to reduce analytical error to a minimum (within-run precision only, no lot-to-lot bias). Long term stability urine for 3 months at least

ing assay technologies are generally not suitable for PCR technologies. And finally, the reflection again made on several occasions at international meetings is that the BMV is really written around a specific CoU too, in other words, PK assays, with the recommendation not to broaden the application of the BMV beyond its CoU.

3. Take-home messages on CoU principles

The EBF recommends that the requester of the BM data, the key stakeholder for the results, rather than the bioanalytical scientist or bioanalytical representative on the team, ensures delivery of the CoU statement to the bioanalytical scientist for every study, and for every analyte in that study. The bioanalytical scientist is not ultimately responsible for the CoU statement but should receive the CoU by default from the requester and from there should be responsible for translation of the CoU into a bioanalytical strategy. Whether it's a clinician, a medical affairs representative, a clinical pharmacology team lead, a BM team lead, or other key stakeholder, each requester should input toward the CoU statement for the bioanalytical scientist or discuss the CoU together with him or her, independent of technology or analyte. In addition, the requester must include a bioanalytical expert in the analysis of published data, so that the team understands how the BM assay described in a publication, and then referred to, was characterized, and whether it can be discerned if this characterization was done appropriately. This CoU statement should be reviewed as a project moves forward, and updated if needed following the same principles, to include an aligned understanding of any changes of a particular CoU, with variability due to for example, increases in patient populations or expected changes, which are so critical to know. Critical are those changes that might jeopardize the validity of the already used or planned analytical method. If the sponsor for a CRO is unable to provide a CoU statement in full (for example, IP restrictions), the CRO would need to carefully document discussions with the sponsor around the assay's suitability. It would be the responsibility of the sponsor to check and confirm whether the proposed work package and assay choice is suitable in advance, and after characterizing the assay's analytical performance, providing an additional confirmation that the data generated is fit-for-purpose and adequately supports the CoU.

4. Conclusion

We propose here a format for the CoU statement which teams can use for every type of BM assay request. The EBF continues to recommend that the real CoU statement is used as the basis for developing and validating BM assays for generating BM data, and not misapplying guidelines for another CoU, the measuring of drug concentrations. Without the CoU statement, inappropriate acceptance criteria, poor use of resources and time and wrong decisions can happen, which in turn could lead to failed drug development, ultimately negatively impacting patients. CoU must be re-evaluated in an iterative approach as the purpose of the BM data changes, and this will dictate assay selection, characterization and much later validation and acceptance criteria. Documentation of the CoU throughout the lifecycle of each BM assay in method summaries, validation plans and validation reports is essential, because the purpose of the assay may change from one study to the next, the types of decisions being made based on the results may vary and should be communicated each time and institutional knowledge may change. Without an agreed CoU there is a risk of implementing the wrong assay, with inappropriate characterizations and therefore validation and acceptance criteria. As a result of presenting this recommended CoU statement format, the EBF hopes that this helps the bioanalytical and drug development community in the implementation of CoU principles to better ensure the discovery and development of safe and effective drugs for patients. At the same time, and equally urgent, we want to stimulate an open discussion with regulators and extricate the expectations for BM assay validation from the limitations of the (often scientifically incorrect) harness of PK assay criteria.

5. Future perspective

What we have learned over the past year is that cross-functional communication between stakeholders remains key to delivering the appropriate BM data to support scientifically strong drug discovery and development strategies. BM strategies must contain, for each analyte going forward, the CoU statement for each analyte as early as possible, and the team discussions on this are a continual, iterative process. Through the EBF-driven, locally situated Roadshows, we have learned that this CoU statement must come from the requester, either the team key stakeholder or sponsor if the assay is outsourced to a CRO. As such, the CoU statement is a simple definition that can be implemented early in the lifecycle of a BM strategy, to effectively support the generation of key BM data from bioanalytical scientists. And reporting of that data as being generated from a validated assay, appropriately validated according to the purpose or CoU of that assay, should be the language that is used going forward for organizations, whether they are validated in pharmaceutical, biotech, or CRO companies and regardless of the purpose of the assay, including when *in vitro* diagnostic regulations might be in consideration. And finally, organizations should be careful to not overextend the CoU statement, to be sure that the information provided is enough to help the bioanalytical scientist to determine how the BM should be measured.

The EBF community also sees the need to apply CoU principles across all bioanalytical requests, not only BM assay requests. Through continued exposure to this discussion, the EBF believes that superior quality data relevant to any drug discovery and development program will be generated going forward. Hence, we also invite individual organizations to share and publish their experience with using CoU for BM Assay validation, and share the risks they have experienced when using BMV for PK assays (i.e. FDA BMV guidance [16] or ICH M10 guideline [17]) for BM assay validation. And that this can only benefit patients and their physicians.

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Disclaimer

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