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Highlights of 4th Regulated Bioanalysis Workshop

"Discussing, Reviewing, Sharing Perspectives, Providing Potential Solutions and Agreeing upon a Consistent Approach on the Recent Issues in Regulated Bioanalysis"

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Abstract

The 4th Calibration and Validation Group (CVG) Workshop on Recent Issues on Regulated Bioanalysis was held in Montreal, Canada on 22 -23 March 2010 for a two-day full-immersion workshop for pharmaceutical companies, regulatory agencies, and contract research labs from around the globe to discuss very current bioanalytical and regulatory challenges. A consensus was reached among the panelists and attendees, which exceeded 300, on key points regarding bioanalytical method validation of both small and macromolecules, in addition to newer and significant technologies and methodologies including immunoassays, biomarkers, metabolism, and dried blood spot analysis. The panel and attendees represented major players in the industry with primary responsibilities in bioanalysis from all reaches of the planet, and almost every author of recent White Papers and Crystal City consensus papers were present, including C.T. Viswanathan, PhD. Despite there being consensus made on numerous bioanalytical issues that impact scientists and auditors, the panel and audience rallied to resolve the best course of action for the harmonization of bioanalytical method validation (BMV) guidance: in short, to have a single guidance document for all world agencies to use. Viswanathan reemphasized the plea he made recently at the Brussels meeting for 'globalization' of the guidance. In addition to information presented from EMA, MHRA, Health Canada, EBA and ANVISA, the FDA made new announcements and conveyed issues that it felt were now significant to the industry and under consideration for the revision of the method validation guidance that the FDA is targeting for distribution, hopefully by early 2011. The accompanying text is a summary prepared by a member of the SQA BioAnalytical Specialty Section, Chris Tudan, PhD, describing the presentations, announcements, consensus and BMV harmonization perspectives. Because the CVG will publish a White Paper pertaining to the meeting (due January 2011), this document is meant only to be a summary written for the members of the SQA, and was not formatted as a journal manuscript.

Introduction

On 22 -23 March 2010, the Calibration and Validation Group (CVG) and Canadian LC-MS Group organized and held in Montreal, Canada, the 4th Regulated Bioanalytical Workshop. These meetings have become an instrumental arena for global regulatory and pharmaceutical representatives to discuss and develop consensus on issues as they apply to bioanalysis in the pharmaceutical industry. Recently, regulatory bioanalysis has become the centerpiece for

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discussions, while maintaining a non-prescriptive scientific foundation of dialogue and decision making. The 2010 4th Regulated Bioanalysis Workshop was one of a number of predetermined global meetings emphasizing the need for harmonization, or more accurately, globalization of bioanalytical method validation guidance (BMV). These meetings now have immediate importance given the real-time globalization of the industry, currently without real or relevant guidance, and the introduction of the EMA draft guidance of BMV and revision of the FDA version that is due next year.

The CVG is a Canadian-based, scientific organization that partners with industrial, academic and regulatory bodies to provide education and forums for discussion on calibration and validation practices within the pharmaceutical community. The collaboration of the CVG with the Canadian LC-MS Group has resulted in published White Papers from the 2008 and 2009 meetings. These organizations, during these meetings and in conjunction with the European Bioanalytical Forum (EBF), were a leading part of the global initiative for clarity on bioanalytical guidance's that can be applicable. The links to these White papers are:

- 2008 White Paper: http://www.future-science.com/doi/pdfplus/10.4155/bio.09.11
- 2009 White Paper: http://www.future-science.com/doi/pdfplus/10.4155/bio.09.134

A more succinct history of this can also be found in the following references:

- Bansal, K., Arnold, M., and Garofolo, F. (2010). International harmonization of bioanalytical guidance. *Bioanalysis*, *2* (4), 685-687.
- van Amsterdam et al. (2010). Towards harmonized regulations for bioanalysis: moving forward. *Bioanalysis, 2* (4) 689-691.

The meeting was hosted by Fabio Garofolo, PhD, who is the founder and president of the Canadian LC-MS Group and Vice President, Bioanalytical Services of Algorithme Pharma. Garofolo gave an excellent introduction and chaired the consensus discussions. He introduced the speakers who also comprised the consensus panel, and introduced the "Hot Topics" around which the panel and attendees discussed and derived consensus. These topics included the following:

- Bioanalysis and method validation harmonization
- Lipemic and hemolyzed samples – not reportable or additional development required?
- Statistical challenge to current validation criteria
- Urine and tissue analysis
- Repeat analysis of multi-analyte assays and failed runs
- Preparation of calibration standards
- LBA critical reagent stability
- Sample handling
- Endogenous analyte assays
- Carryover criteria

The bioanalytical industry is governed by enacted laws, and regulations issued under those laws, by regulatory agencies of many different countries. Although these regulatory agencies often provide suggestions of practices that they will generally accept under these laws and regulations, the status quo for bioanalysis remains that of the FDA 2001 guidance and the Crystal City III White Paper. These documents, and that of the MHRA (2009), EMA draft (2009-2010) and BQSI (2009), are written in general terms, leaving agencies and the specifics of execution up to the industry. Since the industry is faced with the fast-paced evolution of technology, new drug paradigms and regulatory requirements, these guidance documents are not current and thus cannot reflect the current bioanalytical climate. Therefore the agencies are using workshops such as this to delineate new, and clarify old expectations. At the 2010 Workshop, the following individuals provided regulatory updates and perspectives on BMV harmonization:

- Brian Booth, PhD, US FDA
- CT Viswanathan, PhD, US FDA
- Louise Mawer, U.K. MHRA
- Jan Welink, PhD, EMA representative

- Eric Ormsby, Health Canada
- Arthur Leonardo Lopes de Silva, Brazil ANVISA
- Peter van Amsterdam, PhD, EBF

These communications are discussed in this summary. When I consider the Workshop now, there are some considerations that I wish to emphasize to preclude your reading this summary. In as much as the globalization of bioanalysis has been driven by the scientific community, it was conveyed at the Workshop that globally harmonized instructions should be of equal interest to both the regulators and practitioners of bioanalysis. Bioanalysis provides essential regulatory data for TK, PK, BA and BE studies, and relevant data are consistently submitted to various regulatory agencies around the world. Furthermore, both science and regulations have co-existed for many years, and both have progressed and transformed over the years. Therefore, both science and regulations may have influenced each other, but each has its individual development pathway. For this reason, it is my hope that the SQA wastes no time in becoming involved in the endeavor of BMV harmonization. Some points to consider for which there was no consensus included the following questions:

- What would be the global regulatory guidance?
- Publication type (OECD or ICH)?
- What other regulatory guidance/regulations equally impact bioanalytical work, for instance:
    - GLP and GCP;
    - Analytical instrument qualification; and
    - Managing and archiving of electronic data?

The consensus descriptions within are derived from the slides presented at the meeting. I prepared this meeting summary based on notes taken by me, since electronic copies of the presentations were not available at the time. I hope this information is of value to you, and timely, and I am receptive to feedback and dialogue.

Presentations

Fabio Garofolo, PhD (VP Bioanalytical Services, Algorithme Pharma)

Summarized 2009 Meeting and associated White Paper [2009 White Paper on Recent Issues in Regulated Bioanalysis from the 3rd Calibration and Validation Group Workshop – Savoie et al., (2010) Bioanalysis 2(1), 53-68].

The 2009 meeting was focused on reuniting the bioanalytical community to exchange knowledge, recent perspectives on bioanalytical issues and regulatory challenges faced by the bioanalytical community, and the focus was primarily small molecules.

Key issues described and consensus obtained in the 2009 meeting and published in the 2010 paper were:

1. Manually integrated chromatograms
2. Impact of the presence of metabolites on quantitation
3. Effect of hemolysis
4. Procedure for investigation
5. Anticoagulant used in the study – Must be consistent with the validation, otherwise additional testing is required; namely, the anticoagulant must be consistent between the validation and the sample analysis.
6. Blood Stability testing – A collection process stability experiment that should be performed during method validation to ensure method integrity during sample analysis.
7. Ion Suppression and Matrix effects – Best to correct by using a stable-labeled internal standard, or less ideally, by reducing the flow rates and/or use of smaller ID HLPC columns (such as 1mm).
8. Assessing Contamination – The consensus was to maintain the 20% LLOQ criteria, but there was no consensus on how to determine the impact of carryover. The later will be assessed during the 2010 meeting.
9. Non-linear calibration models – Meeting consensus was to use a quadratic fit for a large dynamic range and potential linearity problems should never be masked. It was conveyed that no 483s have been reported for using a quadratic fitting model throughout an entire study.

10. The 2009 ASMS Regulated Bioanalysis Interest Group Workshop (RBIG) is posted on the ASMA Regulated Bioanalysis Forum at www.asms.org.
11. The 2009 APA Conference report is available at: Ackerman (2010). Conference Report: Applied Pharmaceutical Analysis 2009 Conference. *Bioanalysis, 2* (2), 185-188.

The 2010 introduction by Garofolo highlighted a number of important documents introduced in 2009, starting with a discussion of the Bioanalytical Quality Standard Initiative (BQSI) guidance document that was submitted to the FDA as a level 1 guidance document in 2009 (Docket No. FDA-2009-D-0428). The Guidance document was titled: "Quality Management System for Bioanalysis Supporting Clinical Trials." The mandate of the BQSI in developing this document was to develop guidance for a Quality Management System to be used by laboratories conducting bioanalysis supporting clinical trials.

Note that concurrently, the MHRA issued: "Guidance on the Maintenance of Regulatory Compliance in Laboratories that Perform the Analysis or Evaluation of Clinical Trial Samples." This was issued in July 2009.

The EMA (European Medicines Agency) and Committee for Medicinal Products for Human Use (CHMP) released for consultation in November 2009 a Draft Guideline on Validation of Bioanalytical Methods.

"Draft Guidance of Validation of Bioanalytical Methods"

Link: http://www.ema.europa.eu/pdfs/human/regaffair/2414304en.pdf

Released for Consultation: November 2010
Deadline for comments: 31 May 2010

Although it was developed to contain recommendations for a guidance for the validation of bioanalytical methods, which has been lacking in the EU community, and parallels many of the guidance's conveyed in the FDA May 2001 document, there are some major differences, for instance, as outlined below.

Subject	EMA	FDA
Reference Standards	Discusses isotopic expectations of labeled reference standards	No reference to isotopically labeled reference standards
Selectivity	Response of interference is < 20% of LLOQ	No specific criteria
Selectivity	Includes tests for possible metabolic back-conversion	No specific tests recommended
Recovery	Not discussed	Required
ISR	Required; criteria provided	Not formally discussed in guidance, but enforced by FDA and described in Crystal City III conference paper
Carryover	Required	Not discussed
Matrix Effect	Discussed specifics of evaluation and criteria	General statement that it should be investigated
Stability	Provides criteria of 15% from nominal concentration	No specific criteria provided
PK Outliers	Not recommended	Allowed

Garofolo introduced the ten questions, or "Hot Topics" that were selected for the 2010 White Paper. The first, and really the primary, topic of the meeting was that regarding Bioanalytical Method Validation Harmonization. All representatives agreed that because the FDA BA May 2001 guidance has been accepted industry wide since

its release, and because of the globalization of the drug market, it is in everybody's interest to harmonize the requirements.

Three editorials and one open letter have recently been published strongly recommending the harmonization of bioanalytical method validation, and a representative document thereof. Briefly, these include:

1. van Amsterdam et al. (2009). European Bioanalysis Forum and the way forward towards harmonized regulations. *Bioanalysis 1* (5), 873-875
2. Bansal, K., Arnold, M., and Garofolo, F. (2010). International harmonization of bioanalytical guidance. *Bioanalysis 2* (4), 685-687
3. van Amsterdam et al. (2010). Towards harmonized regulations for bioanalysis: moving forward. *Bioanalysis 2* (4), 689-691.
4. Timmerman, P., Lowes, S., Fast, D. M., and Garofolo, F. (2010). Request for global harmonization of the guidance for bioanalytical method validation and sample analysis. *Bioanalysis 2* (4), 683.

Consensus was made during the meeting and will be the topics of the 2010 White Paper. These are summarized in detail at the end of this document.

Christopher Evans, PhD (US Head, Bioanalytical Science & Development, GlaxoSmithKline) - "Use of dried blood spots (DBS) in regulated bioanalysis: Practical considerations, applicability, and future directions."

Evans addressed the following questions: (i) Is the DBS technique the future of samples collection? (ii) Could DBS be used as replacement matrix for plasma for supporting drug development in clinical pharmacokinetic studies?

The rebirth of Dried Blood Spots (DBS) technology and the interest in associated regulations considerations is a result of (i) external pressures in the industry to deliver high quality PK data in the shortest possible time, and (ii) to reduce, refine and replace the use of animals in drug development. A key impetus to the last point is the challenge to collect adequate samples while staying within acceptable total blood collection volumes and avoiding excess animal use.

Evans described the techniques and related (and currently limited) technologies associated with this approach to plasma bioanalysis. Some of the advantages of this technique include:

- Addressing the increased requirements for pediatric PK and TK studies
- Reduced sample volumes and processing required
- Simple and room temperature storage requirement
- Reduced sample storage space
- Reduced costs
- Ethical benefits
- Simplified sample collections (finger prick)
- Possible improved recruitment
- Ideal for Phase II/III studies in developing countries
- Advantages for critically ill patients
- Simplified population PK study sampling
- Simplified sample processing (centrifugation, sub-aliquotting)
- Potential for greater compound/metabolite stability

Evans and his colleagues at GlaxoSmithKline have been validating the technique as per the May 2001 guidance, with success, and he discussed how the technology is robust enough to support validated methodologies as per internationally accepted criteria. Furthermore, Evans described how DBS is being implemented within other pharmaceutical companies and CROs. Note that DBS is the key topic of the recent AAPS News Magazine (April 2010).

Eric Woolf, PhD, Senior Director, Merck Research Laboratories, "Bioanalytical Run Assessment - Factors to Consider Beyond the White Paper."

Woolf emphasized with supporting case studies how the evaluation of internal standard (IS) criteria and tracking internal standard response and calibration curve slopes would help support the accuracy of analytical results. By assessing trends in the internal standards response, which he argued should be consistent, potential issues that impact the robustness of the method and/or accuracy of the results can be identified.

Case study data were presented, and it was convincingly argued that calibration curve slope is still important in LC-MS/MS bioanalysis, and in fact, can be used as a test for the assessment of potential issues associated with a method during method development, validation, and sample analysis. An important caveat is that the issues will likely not impact the data, but remaining consistent with Woolf's concluding remark ["As bioanalysts, we are responsible for ensuring that the methods that we develop and utilize are sufficiently accurate and precise to measure the actual concentrations of analyte in the samples we analyze"], method processes and sample preparation (such as internal standard stock solution preparation errors) inconsistencies can be identified so that cause can be appropriately documented, and the impact effectively elucidated.

In summary, internal standard response (IS response review) and calibration curve slope tracking can act as indicators of:

- Relative matrix effects,
- What is actually going on with study samples,
- Insight into operator/instrument issues.

During Woolf's presentation, Dr. Viswanathan approached the microphone and indicated that 21 CFR 320.29 will be revised by the FDA soon.

Mohammed Jemal, PhD, Senior Research Fellow, Bristol-Myers Squibb, "Bioanalytical Method Quality vis-a-vis Method Development, Qualification, Validation and ISR."

Jemal shared his in-depth experience on metabolites quantification and the respective implications on effective method development and validation. The presentation provided technical insights into methodologies to address the metabolite and phospholipid risk avoidance.

Relevant References:

Metabolite Interference:

- Jemal, M., Ouyang, Z., & Xia, Y-Q. (2010). Systematic LC-MS/MS bioanalytical method development that incorporates plasma phospholipids risk avoidance, usage of incurred sample and well thought-out chromatography *Biomedical Chromatography, 24*, 2-19.
- Wu et al. (2009). Distinguishing a phosphate ester prodrug from its isobaric sulfate metabolite by mass spectrometry without the metabolite standard. *Rapid Communications in Mass Spectrometry, 23,* 3107-3113.
- Jemal, M., & Xia. (2006). LC-MS Method development strategies for quantitative bioanalysis. *Current Drug Metabolism, 7*, 491-502.
- Kapron, J. et al. (2005). Removal of metabolite interference during liquid chromatography/tandem mass spectrometry using high-field asymmetric waveform ion mobility spectrometry. *Rapid Communications in Mass Spectrometry, 19,* 1979-1983
- Jemal, M., Ouyang, Z., & Powell, M. (2002). A strategy for a post-method-validation use of incurred biological samples for establishing the acceptability of a liquid chromatography/tandem mass-spectrometric method for quantitation of drugs in biological samples. *Rapid Communications in Mass Spectrometry, 16*, 1538-1547
- Jemal, M. & Xia, Y-Q. (2000). Bioanalytical method validation design for the simultaneous quantitation of

analytes that may undergo interconversion during analysis. *Journal of Pharmaceutical and Biomedical Analysis 22*, 813-827.

Phospholipids Chromatographic Behavior and Detection by MS/MS:

- Xia, Y-Q. & Jemal, M. (2009). Phospholipids in liquid chromatography/mass spectrometry bioanalysis: comparison of three tandem mass spectrometric techniques for monitoring plasma phospholipids, the effect of mobile phase composition on phospholipids elution and the association of phospholipids with matrix effects. *Rapid Communications in Mass Spectrometry, 23,* 2125-2138
- Mess, J. et al. (2009). Selection of HILIC columns to handle matrix effect due to phospholipids. *Bioanalysis 1* (1), 57-62
- Jemal, M., Ouyang, Z., & Xia, Y-Q. (2010) Systematic LC-MS/MS bioanalytical method development that incorporates plasma phospholipids risk avoidance, usage of incurred sample and well thought-out chromatography *Biomedical Chromatography, 24*, 2-19.

Robert Masse, PhD, Vice President, Bioanalytical Division, Anapharm, "A CRO Perspective on ISR: Real Case Examples in an Evolving Regulatory Landscape."

Masse presented topics and case studies on incurred samples reanalysis (ISR), how to investigate ISR failure, and what to do if the parent drug passed ISR but the metabolite failed.

Recommendations regarding global harmonization of bioanalytical validation guidances:

- Provide acceptance criteria for ISR
- Details about the selection of ISR samples
- Define complexity, scope and duration of investigation to address failed ISR assessment.

Key points made pertaining to robust method development and validation:

- Ensure a knowledge of analyte chemistry and metabolism prior to method validation (determined before or during method development),
- The use of a stable-labeled internal standard is highly recommended,
- Identify potential stability issues and resolve them prior to method validation (determined before or during method development),
- Assess the potential impact of technical procedures on assay performance.

The assessment of technical procedures is particularly relevant to ligand binding assays. Although this was not conveyed during Masse's presentation, this was discussed during the meeting. Relevance is pertinent to immune-assay types, labeling and instrumentation, where the selection can impact sensitivity, selectivity, LOD and curve range.

Mario Rocci, Jr, Executive VP, ICON Development Solutions, "Investigation and Resolution of Incurred Sample Reanalysis Failures - Two Case Studies."

Rocci discussed two case studies that demonstrate ISR failures and the investigation preformed with each that identified[1]:

- The root cause of the ISR failure
- Corrective actions to circumvent the respective cause.

Marc Lefebvre, PhD, VP Scientific & Regulatory Affairs, Algorithme Pharma, "Impact of Bioequivalence/PK Study Design on the Development of Bioanalytical Methods."

Lefebvre spoke about the recent updates in biostatistical analysis in bioequivalence/PK studies. Lefebvre made the

1 Fast et al. (2009). Workshop Report and Follow-Up , AAPS Workshop on Current Topics in GLP Bioanalysis: Assay Reproducibility for Incurred Samples – Implications of Crystal City recommendations. *The AAPS Journal 11* (2), 238-241

following conclusion that has significant relevance to how technologies and drug paradigms have advanced enough to justify the harmonization of method development and validation approaches, and communications thereof is important today:

- The development and validation of bioanalytical methods are more complicated in order to meet agency's data;
- With the commercialization of more powerful drugs, doses are lower, and PK profiles are difficult to determine;
- With the commercialization of new modified-release formulations, dosing and PK profiles are being modified;
- We always try to develop only one specific method for a drug, but it is regularly updated due to different study designs, study formulations, etc.;
- Overall, the development and validation of new bioanalytical methods are more and more a team effort where communication is the key factor.

Stephen Lowes, PhD, Senior Vice President Scientific, Advion BioServices, "Bioanalytical Considerations for Development of Regulatory Guidance around LC-MS Biomarker Assays."

Lowes lectured on the technical challenges in providing quantitative analysis in a regulated environment for Biomarkers and on the development of a Regulatory Guidance able to meet the growing industry demand in this field.

Lowes gave an excellent presentation pertaining to biomarkers. Although centered on assessing biomarkers via LC-MS/MS, his topic enveloped the importance of this bioanalytical paradigm as a whole. Lowes' presentation sparked a lot of discussion around the need for non-prescriptive regulatory consensus and a serious consideration of having a section within the new FDA guidance document for biomarkers.

Lowes' presentation addressed the regulatory challenges associated with biomarkers that are distinct from the measurement of xenobiotics. Bioanalytical challenges that make biomarker analyte quantitation unique include:

- Endogenous to sample,
- Subject to complicated biological system variables,
- Comprise a wide range of species from simple small molecules to large biomolecules (lipids and proteins),
- Present in a whole range of biological matrices,
- Pertinent concentrations may fluctuate to a small degree over a wide dynamic range.

Therefore, if biomarker assays are to be bound by regulatory guidance's, then the unique attributes of biomarker analytical challenges must be taken into account. Remaining non-prescriptive with such guidelines becomes more significant in that depending upon how the data are to be used, method validation or various levels of method qualification need to be considered.

This topic is of interest to the FDA. Furthermore, since it is clear to representatives at CDER that the definition of method validation versus qualification is not universally understood (as can be verified in recent 483s), and new guidance document that describes bioamarkers will likely clarify the definitions of quantitative validation and non-quantitative qualifications, or quantitative qualifications that cannot be validated, for instance in the case of urine matrices, etc. (Opinion of C. Tudan). Lowes presented a 'Dual Step Decision Process' to help in the method categorization decision process making.

Louise Mawer, Senior GCP Inspector, U.K. MHRA, "Considerations on Follow-on Biologics and Biosimilars." Mawer shared the Agency considerations pertaining to follow-on biologics and biosimilars.

Patrick Bedford, Senior Policy Analyst, Office of Policy and International Collaboration, Biologics and Genetic Therapies Directorate, Health Canada, "Health Canada's Guidance for Subsequent Entry Biologics."

Bedford spoke on Health Canada's Guidance for Subsequent Entry Biologics. This presentation was more historical, but the emphasis was that Canada was now initiating OECD practices and laboratory certification.

Joseph Marini, PhD, Associate Director, Centocor Research & Development, Johnson & Johnson, "Ligand Binding Assay Validation and Bioanalysis: Challenges and Solutions."

Marini focused on LBA recent and old challenges and solutions, discussing the validation of large molecules: specificity, selectivity and non linear calibration, as well as new technologies available (pros and cons) and the new White Paper.

Joleen White, PhD, Senior Research Investigator, PCO-BioAnalytical Sciences, Biologics, Bristol-Myers Squibb, "The Value of Including Incurred Samples in Immunoassay Cross-Validation."

White also focused on LBA Recent and old Challenges and Solutions, including:

- Validation of large molecules: specificity, selectivity and non linear calibration;
- LBA Free/Total; LBA new technologies available: pros and cons;
- Orthogonal methods to complement LBA for biotherapeutics and why do we need them?
- Neutralization assay: New White Paper.

Peter van Amsterdam, PhD, Head of Global Bioanalytics, Abbott, "EBF Perspective on the new EMA guidelines on bioanalytical method validation (BMV) and input on global BMV harmonization."

van Amsterdam shared the EBF (European Bioanalysis Forum) input on global BMV harmonization.

The EBF is an EU based organization of bioanalytical scientists that work within the pharma industry and R&D. Founded in 2006 upon the initiative of 12 pharma companies with bioanalytical laboratory activities in the European Union, the EBF now consists of 27 member companies. The EBF has biannual closed meetings to discuss:

- Regulatory issues and aspects
- Share common practices on procedures
- Science
- Electronic Systems
- Validation
- Quality (GLP)
- Reporting
- New developments in industry

The EBF also organizes conferences for the bioanalytical community for discussions regarding challenging procedures and techniques as well as new regulatory requirements.

A summary of their positions and activities can best be illustrated by three slides from their web site (http://www.europeanbioanalysisforum.eu/):

Networking / Collaborations:

- AAPS / APA / CVG on BMV harmonization
- EUFEPS on EMA BMV
- EIP on bioanalytics re: immunogenicity
- International Reid Bioanalytical Forum
- Vision to set-up:
  - World/Global/International
  - Bioanalytical
  - Council/Congress/Federation

Publications (paper):

- Letter to authorities on BMV harmonization (April 2010)
- Editorial in "Bioanalysis" on BMV harmonization (April 2010)
- EBF 2nd open conference paper (April 2010)
- Qualified assay / tiered approach to validation
- MIST tiered approach
- EBF-EUFEPS meeting conference report (April 2010)
- EBF-DBS meeting conference report (Jun 2010)
- Coagulant of choice / counter ions

Comments on Guidelines:

- Consolidated EBF-IGM comments on FDA draft guideline on immunogenicity (January 2010)
- Consolidated EBF comments on EMA draft guideline on BMV (May 2010)

Opinions pertaining BMV guidance's and harmonization:

The FDA guideline and Crystal City III report is the "Gold Standard."

There is some anxiety within the bioanalytical community that there may be two guidances (FDA/EMA) with contradictory content. Because there is a belief that there are emerging economies entering the field of regulated BA and belief that a global harmonized guideline is the best way forward, the EBF is supporting a willingness at FDA and EMA to work towards a harmonized guidance based on their respective documents.

The harmonization process can be lengthy, and the EBF feel that the sooner the process starts, the better.

The industry is trying to gain input and consensus upon which umbrella a harmonized guideline should be under: ICH, OECD, WHO, or BRIC?

EBF wishes to work jointly with AAPS, BFG, APA, CVG, LC-MS Groups. They sent a letter to C.T. Viswanathan (FDA) and P. Le Courtois (EMA) on 12 February 2010:

- Describing the landscape
- Acknowledging continued globalization of pharma industry and thus bioanalysis
- Expressed a need for uniformity in the guidelines

Dried Blood Spots: Although there is plenty of room for improvement and technical advances, there is enough experience to begin addressing an emerging regulatory acceptance for blood versus plasma PK.
Key Topics were expressed by van Amsterdam describing EBF positions on hot topics in BMV:

1. Matrix Effect
   a. The EBF feels the definition of matrix effect should be expanded to include binding assays and methodologies to address them.
2. Study Report
   a. It is preferred that neither SOPs or chromatograms in searchable PDF formats be attached to the report,
   b. Confused with CCIII report versus FDA guidance,
   c. Likely a difference with BE studies.
3. Legal Basis
   a. Avoid any reference to GLP unless GLP compliance is required,
   b. How is GLP enforced, especially with a global document? How are they enforced?
   c. Propose running validations according to SOPs in GxP compliant facilities (GxP accreditation).

4. ISR
    a. Members of the EBF prefer the implementation of the EBF ISR proposal, and are seeking a clarification on when and how to do ISR (Bioanalysis 2009 1:6, 1049-1056).
5. Stability
    a. Better definitions on partial and cross validations,
    b. Stability data should be available when the study results are reported,
    c. Blood stability investigation should be part of the method development as opposed to the validation,
    d. Agreement with the EMA believes that Incurred Sample Stability needs to be evaluated,
    e. Acceptance criteria should be based on nominal versus a mean or T0 (line ISR concept).
6. Accuracy
    a. Inter- and intra-assay accuracy is not meaningful and does not fully cover the calibrated range. Should be over the entire study,
    b. Determination of an outlier should be written in an SOP or Protocol a priori to method analysis and validation.

Surendra Bansal, PhD, Research Director Bioanalytical R&D, Non-Clinical Safety, Hoffmann-La Roche, "International Harmonization of Bioanalytical Guidance."

Bansal discussed international harmonization of the bioanalytical guidance as a serious of discussions, (i) Scope, (ii) Historical Perspective, (iii) Global bioanalytical and bioanalytical guidance landscape, (iv) Current activities/articles and (v) a summary and future steps.

Bioanalysis provides essential regulatory data for TK, PK, BA and BE studies, and relevant data is consistently submitted to various regulatory agencies around the world. Furthermore, both science and regulations have co-existed for many years, and both have progressed and transformed over the years.

Therefore: Science and Regulations may have influenced each other, but each have their individual development pathway.

Bansal noted that bioanalysts have been following the 2001 Guidance and Crystal City III reports religiously to avoid any uncertainty, even though not following the guidance was acceptable so long an a priori justification is given to the alternative approach. It is worth noting that there was agreement that the bioanalytical community simply followed the guidance and consensus papers although the FDA purposefully wrote the guidance to allow for scientific flexibility.

The current bioanalytical landscape is ripe for harmonization of a BMV guidance document. Key aspects if this landscape includes:

- Bioanalysis is performed on a global basis
- Global CROs and Pharmaceutical companies
- Analysis performed in one country is usually also submitted to other(s)
- Regulatory agencies are performing global inspections
    - Regulation is not limited to national borders
- Most guidances reference the FDA guidance and Crystal City conference reports. Although there are multiple guidances out there, limited comprehensive guidance still remains.
- Regulatory guidances are supplemented with conference reports that have equal weight in guiding bioanalysis (and source for 483s).

When considering future harmonization, it was recognized that other regulatory guidances/regulations can equally impact bioanalytical work, for instance:

- GLP and GCP,
- Analytical Instrument Qualification,
- Managing and archiving of electronic data,
- Part 11, Documentation procedures and content (C. Tudan).

Consensus at the meeting is that for the sake of time, harmonization should start with the bioanalytical guidance.

In as much as the globalization of bioanalysis has been driven by the scientific community, it was conveyed that globally harmonized instructions should be of equal interest to both the regulators and practitioners of bioanalysis.

Bansal raised some big questions that require consideration, namely:
- What would be the global regulatory guidance?
- Publication type (OECD or ICH)?

A globalized BMV document could also consider a globalization of method "documentation" such that a similar format of reporting is also common globally (C Tudan).

Global Summary Discussion

Brian Booth, PhD (USA-FDA), C.T. Viswanathan, PhD (USA-FDA), Louise Mawer (UK-MHRA), Arthur Leonardo Lopes da Silva (Brazil-ANVISA), Jan Welink, PhD (Europe-EMA representative / Dutch Medicines Evaluation Board) and Eric Ormsby (Health Canada) were engaged in a stimulating and interactive discussion on Global Harmonization of Bioanalytical Method Validation Guidelines (BMV) and Sample Analysis. This worldwide important topic was introduced by Surendra Bansal, PhD (Hoffmann-La Roche).

Brian Booth, PhD, Deputy Director, Office of Clinical Pharmacology, Center for Drug Evaluation and Research, US FDA and, C.T. Viswanathan, Associate Director, Division of Scientific Investigations, Office of Compliance, Center for Drug Evaluation and Research, US FDA - "The FDA Bioanalytical Guidance Revision - Will There be Harmony?"

Guidance Updates:

LBA Issues:
- LBA assay acceptance criteria – 20/25% with total error of ±30% (±40% LLOQ)
- 75% of Standards ±20% (±25% at LLOQ); 4/6/20 rule.

Chromatographic Issue:
- QCs to represent 5% of samples, and spanning the dynamic range
- Partial validations as needed
- Stability should mimic actual conditions of storage and until there is consensus, -20ºC freezing conditions do not mimic -70ºC.

General Issues:
- Tiered approach to novel metabolites
- ISR – To follow the White Paper: Fast et al. (2009). Workshop Report and Follow-Up , AAPS Workshop on Current Topics in GLP Bioanalysis: Assay Reproducibility for Incurred Samples – Implications of Crystal City recommendations. *The AAPS Journal 11* (2), 238-241
- Documentation:
  - Tabulation listing of rejected runs, QC results, reanalyzed samples, ISR
  - Retention/submission of chromatograms

New Issues:
- Clarifications:
  - Define simplest fit, best curve fit
  - Anchor points
  - Stability definition
  - Standard and QC placement
  - Repeat analysis

- Biomarkers
  - Fit for purpose
  - Biomarkers used from target selection to pivotal studies (surrogates)
  - What is the appropriate level of validation needed at each point?
  - Realize that biomarkers now consider the issue of study endpoints!
  - Need an assessment of safety and effectiveness
  - FDA is considering the incorporation of a section in the new guidance to accommodate biomarkers.
  - Immuno-assays and LC/MS based.
- Define Scope:
  - Not qualification
  - Not diagnostic Test development
  - Measurement of study endpoints

According to Viswanathan, the guidance revision has begun, with the hope to finish in 2011. The FDA does not want to be prescriptive in the development of the revised guidance. The FDA wants to give the power to companies and bioanalysts, and this is deliberate.

Additional considerations for the Guidance will include:
- Biomarkers
- Clarifications
- Microsampling/Dried Blood Spot
- Endogenous compounds
- Diagnostic kits
- Electronic data capture [The FDA is now looking for pilot data during audits and will be keying into Part 11]
- Considerations of (i) anticoagulants, (ii) sexes/species, (iii) new instruments, (iv) -20ºC/-70ºC stability clarification.
- EMA document

Harmonization:
It is an FDA opinion that BMV guidance harmonization is desirable and that the FDA and the EMA should work together. It is important to consider how to get to harmonization so what is correct can be achieved in a reasonable amount of time. Moreover, representation for harmonization should to be global approach to be effective and timely (not necessarily the opinion of the FDA, but it is of C.T. Viswanathan).
Discussions during this presentation included:
- The need to form a consortium and to be focused on what is on the table now,
- Globalization versus harmonization is a better focus for speed,
- To have a global consortium of experts and representatives,
- The global consortium should submit simultaneously,
- Do not need lots of meetings, but get to the finished product in a way that it is simple, focused, unified and global.

Viswanathan suggested: “Keep it simple, focused, unified and global.”

Arthur Leonardo Lopes de Silva, Specialist in Regulation and Sanitary Monitoring, Bioequivalence Coordination (COBIO), Brazil ANVISA - “The ANVISA Bioanalytical Method Validation Guidance and its Global Harmonization; Support of Submission in Brazil; CRO’s Certification and Bioequivalence Guidelines.”

De Silva presented the criteria associated with the ANVISA guidance and highlighted differences between the ANVISA document and the FDA and EMA documents.

Jan Welink, PhD, Senior Pharmacokinetic Assessor, Europe-EMA representative / Dutch Medicines Evaluation Board - “A Perspective on the EMA Bioanalytical Method Validation; Status and Introduction of the New Draft EMA Bioanalytical Guideline and View on Harmonization.”

Eric Ormsby, Manager, Office of Science, Health Canada Therapeutic Product Directorate - "The Health Canada Bioanalytical Guidance and Possible Harmonization; Status of the New Health Canada Bioequivalence Guidelines; Foreign Study Submission issues; and GLP/GCP Canadian Certification."

Louise Mawer, Senior GCP Inspector, U.K. MHRA - "A Perspective on Inspections that may Include Bioequivalence Studies; UK GLP and GCP Risk-based Inspections and Phase I Accreditation."

Questions and Answers Period - Consensus

Panel Discussions and Consensus for the 2010 White Paper:

1. **Global Harmonization of Bioanalytical Method Validation and Sample Analysis:** Is harmonization possible? What is the best/fastest way to achieve it? ICH Guidelines or OECD Guidelines or a Bioanalytical World Congress with the active participation of both industry and regulatory agencies?

   Consensus:
   Global harmonization should be science driven and include rationale behind each requirement. The committee and Viswanathan do not recommend a prescriptive guidance. It is important to look at the global picture, not the issues. It will be necessary to obtain buy-in from all countries: namely, by creating an all inclusive consortium (BSAT, AAPS, CVG, EBF, etc.) with worldwide influence to merge FDA and EMA guidance to create a unified guidance.

   It was acknowledged that there are many groups around the world that could get involved. The first step is to reach out to many different agencies (AAPS, CVG, EBF, BSAT) and then form a consortium to create one, unified document that can be presented to the decision makers in each country.

   Global guidance should be science drive and not prescriptive. A suggested plan is to:
   1. Consensus of which documents to move forward;
   2. Combine ideas of these into a single document to see and address what is missing;
   3. Prepare the global document.

   Quotations:

   C.T. Viswanathan:
   "The moment is here for globalization. To have 10 SOPs for one procedure is cruelty."
   "Get momentum. There is a need for one harmonized document."

   Steve Lowes, PhD:
   "We need the voice of membership on this. The AAPS is involved in programming but needs to reach out to everybody."

2. **Lipemic and hemolyzed plasma samples**. Is it allowed to just define these samples as "Not Reportable" values or further method development is needed to analyze them?

   Consensus:
   Heme: For known blood: plasma partitioning issues where the drug favors red blood cell binding, the hemolysis should be done. Physiological properties should be taken into consideration and partitioning constant often available to determine the impact during method development. A robust method will accommodate hemolysis and therefore, hemolysis usually should not require monitoring.

Lipemic: Should not be an issue because stable-labeled IS should generally compensate for any variations. Any affect is simply a matrix effect from the lipids. Furthermore, it is difficult to visually differentiate lipemic samples from others. It was recommended that solid phase extraction not be used to remove lipids during sample preparation because of the particle size in the cartridges. Although there is not a reliable test to consistently measure lipid content in samples, many labs are still doing it. If extraction removes the lipids or they are chromatographically resolved (separated), then a test is not necessary.

Avoid "check boxes". Need to make decisions based on science.

Discussions:

- Any lipemic effect is simply a matrix effect. This needs to be addressed in the method!
- Stable-labeled IS should generally compensate for variations (99% of the time).
- Hemolysis is an EMA Draft Guidance issue. We should comment that this should not be as it is a bioanalytical issue.
- Although the lipemic data is not significant to the bioanalytical method once a method is developed adequately, never ignore the bioanalytical data, but address it with science.
- SPE is not the best method for lipemic samples.
- Avoid "check boxes". Need to make decisions based on science.

3. **Statistical challenge to the current validation criteria** (recent literature). How well does pre-study validation predict the quality of individual incurred sample results?

Consensus:

The guidance document should not be too prescriptive by recommending one method of acceptance criteria over another. Furthermore, method robustness cannot be determined with the 4/6/20 rule, or other statistical evaluations until incurred samples are tested during sample analysis. Nonetheless, the committee and attendees agreed that adding statistical evaluation usually provides assertions of confidence and usually does not improve the 4/6/20 acceptance criteria in terms of allowing for confidence in the integrity of the data resulting from the applicable method.

There is a reference describing alternative methods of statistical analysis for bioanalysis.

4. **Urine and tissues analysis**: Tissue analysis is not controlled until the sample is homogenized and urine analysis is not controlled during sample collection and sampling. What is your approach?

Consensus:

Tissues: Tissue methods should not be considered validated since stability data is not possible to acquire. Should be considered 'Qualified' and held to the highest standard. Generally, since sample collection is not highly regulated, tissue bioanalysis cannot be highly regulated.
Urine: Collection is often overused, and the validation of urine matrix methods should be investigated on a case-by-case basis. If urine data are to be primary data, then collection needs to be regulated.

Discussions:

- It comes down to the stability of the collection analysis. If collection of the matrix can't be regulated, how can one regulate the analytical method?
- When trying to determine how or if the method is to be validated, as opposed to qualified, it is best to consider what the data is being used for. This approach, and that of 'fit-for-purpose,' resonated throughout the meeting.
- Remember, that regarding tissue method, it is possible to get stability of a homogenate.
- Many at the meeting felt that considering the end-point and the issue of sample collection, validating these methods is 'overkill.'

5. **Multi analyte assay, repeat analysis and failed runs:** If repeating for failure on one analyte, do we need to regress and report repeat analytes concentrations that passed previously? Where does an assignable cause for failed runs finish and a non-assignable cause start?

   Consensus:
   One only needs to repeat the original data. Do not regress repeat data. Note that it is acceptable to consider using repeat data for ISR.

   Discussions:
   - It is important to have a priori description in an SOP.
   - When repeating for an analytical cause, repeat analysis of all analytes. When repeating for cause, reassess the one applicable analyte.
   - The other analyte data may be used for ISR, if the lab prefers.

6. **Preparation of calibration standards:** Prepare in bulk and freeze aliquots versus prepare them fresh. There are pros and cons for both techniques. Often, individual labs choose one over the other as a matter of their practice, not because the assay would require it. What is the best technique to use? Is it possible to agree upon a standard uniform approach?

   Consensus:
   Crystal City III allows for both methods as long as you have long term stability data for any bulk preparations.

   Discussions:
   - Remember that this does not apply to validation stability experiments when it is necessary to use freshly prepared standards.
   - When the question was raised to the attendees, a small percentage still always use freshly prepared curves during sample analysis, and more do so (although less than one-half) do so throughout validation.

7. **Critical reagent stability** and assignment of **expiration date** for large molecule. When conjugates from reference material are produced how do you establish the new expiration date?

   Consensus:
   Retest date is first assigned based on previous history of the method, and stability progress is initiated to determine the ultimate expiration date. If it is a composite reagent, set the retest date at the shortest interval of component parts.

   Published article pertaining to this:
   Rup, B. & O'Hara, D. (2007). Critical ligand binding reagent preparation/selection: When specificity depends on reagents. *AAPS Journal, 9* (2), E148-E155.

8. **Sample Handling:** From receipt to disposal. Regulatory agencies constantly target the sample handling during audits: collection, storage, chain of custody in LIMS system, and sample labeling. How can we improve sample tracking to better meet regulatory agencies' requirements?

   Consensus:
   There must be adequate communication between the clinical facility and the bioanalytical lab so that samples are handled properly from the beginning, especially for sensitive assays. It is important to establish a well controlled procedure. Facilities also need to consider limitations about storage specifications in different parts of the world, and take them into account scientifically and practically, if necessary.

Discussions:

- Key issues are (i) record keeping, (ii) chain of custody and (iii) process control.
- Establish as well controlled procedure, with good documentation and a defined chain of custody.
- When the attendees were questioned about the frozen stability at -20ºC/-80ºC issue, a significantly large percentage indicated that within their facilities conduct stability at both -20ºC/-80ºC.
- An argument for conducting stability at -70ºC even though stability is conducted at -20ºC is stability on dry ice, where some drugs are pH sensitive and CO_2 can affect stability.

9. **Challenges in endogenous analyte assays** (e.g., Vitamins, hormones, coenzymes): What are the implications for regulated biomarker bioanalytical methods? What are the regulatory agency perspectives? Are the bioanalytical method validation guidelines good enough for endogenous analytes?

   Consensus:
   Fit for purpose approach should be taken for method validation. Chain of custody and privacy consent need to be strict, even for biomarker assays.

   Discussions:
   - Decisions must be based on science and usually addressed within the method development process.
   - Fit for purpose is not a "Get out of Jail for Free Card."
   - Regarding Biomarker Assays, the Agency will check to see if the laboratory is analyzing what they say they are analyzing as opposed to what they want to analyze to protect "patient consent."

10. **Carryover criteria**: Is it feasible to perform a sample-by-sample assessment of potential impact if carryover greater than 20% of the LLOQ is suspected? What does this measurement estimate? Are new carryover criteria (i.e., 5% possible contribution from previous injection) accepted by regulatory agencies? What are the industry standards after Crystal City III and the use of non-randomized sequences?

    Consensus:
    Carryover must be addressed during method development and sample analysis by creating a strategic approach to minimize it.

    Discussions:
    - Use scientific rationale to determine the impact of carryover and be transparent in reporting it.
    - If there is more than a three order of magnitude change in dose compared to the dose anticipated, or demonstrated to give a ULOQ level, then there is a good likelihood that there will be carryover.
    - If carryover is experimentally inevitable, then an a priori description of how carryover will be addressed during sample analysis must be described in the method.
    - When there is carryover associated with a method, it is important to 'non-random' sample ordering when setting up sample analysis runs.
    - A CRO that is conducting a blinded study with a bioanalytical method that is documented to have carryover should communicate with the client about the associated risk on the integrity of the data if the samples are analyzed randomly. The client should allow the CRO to analyze the samples non-randomly.

Additional Questions Raised and Answered During the Question and Answer Period:

General Comment: "LBA methodology is different, but scientific needs still the same as small molecules. Most small molecule needs apply, so we might just need to widen criteria."

Question: Please discuss LBAs and acceptance or exclusion of standards. Is it acceptable to exclude anchor points or should they be kept in no matter what? What criteria and documentation are required for exclusion of points?

Consensus:

- Anchor points should not be removed to make QCs pass.
- If you have acceptance criteria for anchor points, then it needs to be clearly documented regarding the order of rejection.
- Look at the aggregate deviations of other calibrants. It must be demonstrated that the presence of the anchor point has an effect.

Question: For LBAs, what percentage or minimum number of samples is recommended for ISR? Should ISR be run using the same dilution as was used for the original result?

Consensus:

- Use the same percentage of samples as for small molecules.
- If parallelism evaluations have been done, then the dilution factor should not matter. However, try to use the same factor to limit the number of variables during reassay.

Question: When looking at selectivity of LBAs during validation, what criteria are acceptable for excluding a selectivity sample from the calculation?

Consensus:

- None should be excluded without analytical cause.

Biography of Christopher Tudan, PhD

Chris Tudan, PhD is a regulatory bioanalytical expert who has worked in a variety of bioanalytical labs, developing and validating both ligand-binding, cell-based, PCR and LC-MS/MS assays in the role of both Sponsor and CRO. He is President of BioAccurate Enterprises, Inc., a consulting firm that provides technical and regulatory expertise to the regulatory and drug development community. He also trains biopharmaceutical QAU staff and bioanalytical scientists the science and compliance associated with GLP bioanalysis. Tudan trained as a drug discovery biochemist and has been involved in the development of many assays for discovery and characterization of both small and macromolecules (including peptide memetics), resulting in numerous patents, publications and clinical submissions. He has utilized LC-MS/MS and LBAs throughout his career, developing and validating bioanalytical methods, including GLP-compliant DDI methods. Tudan offers a unique ability to merge the regulations associated with bioanalysis in a GLP environment with the applicable technical and specific method-related details.

If you wish to discuss this meeting further with Chris Tudan, please send comments to bioaccurate@gmail.com.