

Calibration & Validation Group

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**DRAFT**

## **The 2<sup>nd</sup> Workshop on Recent Issues in GLP Bioanalysis**

**April 17-18, 2008, Montreal, Quebec, Canada**

### **WHITE PAPER**

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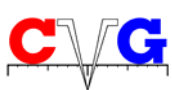
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### **INDEX**

<b>INTRODUCTION.....</b>	<b>Pag.2</b>
<b>ATTENDANCE.....</b>	<b>Pag.2</b>
<b>GOALS AND OBJECTIVES.....</b>	<b>Pag.2</b>
<b>WORKSHOP PRESENTATION SUMMARIES.....</b>	<b>Pag.3</b>
<b>PANEL DISCUSSION.....</b>	<b>Pag.9</b>
<b>ACKNOWLEDGMENTS.....</b>	<b>Pag.16</b>
<b>CONTACT INFORMATION.....</b>	<b>Pag.16</b>
<b>THE 3<sup>rd</sup> WORKSHOP ON RECENT ISSUES IN GLP BIOANALYSIS.....</b>	<b>Pag.16</b>
<b>ACRONYMS.....</b>	<b>Pag.17</b>
<b>FDA DISCLAIMER.....</b>	<b>Pag.17</b>



## Calibration & Validation Group

### **INTRODUCTION**

The 2<sup>nd</sup> Workshop on Recent Issues in GLP Bioanalysis was held on April 17<sup>th</sup> and 18<sup>th</sup> in Montreal (QC), Canada. This event was organized by the Calibration & Validation Group (CVG is a scientific non-profit organization based in Toronto) as a one and a half day full immersion workshop for contract research organizations and pharmaceutical companies involved in providing bioanalytical data for bioavailability, bioequivalence, pharmacokinetic, and comparability studies.

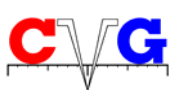
### **ATTENDANCE**

One hundred and thirty nine professionals (scientists, managers, directors, and executives from contract research organizations “CROs”, pharmaceutical companies “Pharmas” and regulatory agencies) from USA, Canada, Europe, Asia, and South America attended this workshop. The attending companies and regulatory agencies were:

Achaogen; Advion Bioservices; Aegera Therapeutics; Agilent Technologies; Algorithme Pharma; Alphora Research; Anapharm; Alta Analytical Laboratory; Angam Systems LLC; Apotex; Applied Biosystems/MDS Sciex; AstraZeneca R&D Montreal; Barr Laboratories; Bausch & Lomb Corporation; Baxter Healthcare; Bayer Crop Science; Biovail Contract Research; BRI Biopharmaceutical Research; Bristol-Myers Squibb; Bruker Daltonics; Cantest; Cardiome Pharma; Celgene Corporation; Centre de Recherche du CHUM; Charles River Laboratories; Chromatographic Specialties; Cobalt Pharmaceuticals; Custom Biologics; Desarrollos Biomedicos Y Biotecnologicos de Mexico; Diteba Research Laboratories; Genpharm ULC; Health Canada – TPD; Hospital General de Mexico; Infinity Pharmaceuticals; Isotechnika; KRKA, d.d.; Lek Pharmaceuticals d.d.; MDS Analytical Technologies; MDS Pharma Services; McGilveray Pharmacon; Merck Frosst Canada; MethylGene; MPI Research; mSPEC Group; Mylan Pharmaceuticals; Neurochem; NHP Laboratories; Nucro-Technics; Pfizer; Pharmascience; PharmOptima; Phenomenex; PPD; Protech Pharmaservices; Quest Pharmaceutical Services; ratiopharm GmbH; ratiopharm Inc.; Ricerca Biosciences LLC; Sandoz Canada; Sosei R&D Limited; Theratechnologies; Thermo Fisher Scientific; Trident Bioanalytics Ltd.; Université de Montreal; US FDA; Varian Canada; Warnex Bioanalytical Services; Waters Canada; Waters Corporation; Waters Mexico; Wyeth Research; YW BioSciences; Zef Scientific.

### **GOALS AND OBJECTIVES**

The 2<sup>nd</sup> Workshop was organized as a follow up to the 1<sup>st</sup> Workshop held in May 2007. (1<sup>st</sup> Workshop Report available on line at [www.cvg.ca](http://www.cvg.ca)). Due to time constraints, the only topic that was covered in sufficient depth at the 1<sup>st</sup> Workshop was Incurred Sample Re-analysis (ISR). Hence, the 2<sup>nd</sup> Workshop continued and expanded the discussions by reviewing, sharing perspectives, and providing potential solutions in order to agree upon



## Calibration & Validation Group

consistent approaches on the main “hot topics”. It also progressed from what has been discussed at the 2007 BSAT-APA Conference, and the recent Feb. 2008 AAPS Workshop.

The topics below were suggested by the audience and CVG members both on-site and online, and thoroughly discussed during the 2<sup>nd</sup> Workshop:

- Matrix effects and hemolysis effect: How and why?
- Ion suppression and matrix effect: Do we need full or partial validation for the same compound in different species?
- Autosampler stability and re-injection reproducibility: Are you still using a fresh curve?
- Are standards being set by 483s and not by consensus, i.e. regulating by 483? Overreaction to avoid future 483s.
- Can storage at -70°C and -80°C be considered equivalent?
- Changing type of anticoagulants: What validation parameters should be evaluated?
- Contamination criteria: What criteria are you using?
- Acceptance of nonlinear calibration models? How much “quadratic” is acceptable?
- What criteria for PK repeats are you using? Forum for discussing how various companies are handling PK repeats.
- What is your approach for incurred samples reanalysis? What has already been done to implement the recommendations of the Crystal City III paper on assay reproducibility? (Practices and techniques that have been used since the publication of the 2006 Crystal City White Paper published on AAPS Journal, and available on line at: <http://www.aapsj.org/view.asp?art=aapsj0901004>)

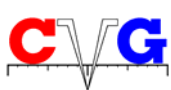
### **WORKSHOP PRESENTATION SUMMARIES**

The followings are summaries of the topics that were presented by recognized experts from regulatory agencies and industry, which were enriched by interactive discussion from the audience.

#### **Good Practices Matter: An Overview in the Trends in GMP, GLP and GCP Use in Regulatory Affairs**

(Dr. Alan Viau, Associate Director, Health Canada-Therapeutic Product Directorate)

The first day of the workshop began with the opening remarks of Dr. Alan Viau, representative of Health Canada – Bureau of Pharmaceutical Sciences. In his presentation, he recognized Health Canada’s need for reviewing their approaches and authorities to protecting the health of Canadians. Due to growing concerns by citizens about the quality and safety of imported food, goods and health products, the agency is proposing targeted actions to modernize the system: active prevention, targeted oversight



## Calibration & Validation Group

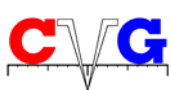
and rapid response. However, it is essential that these actions achieve a balance between fast access, innovation, sovereign/economic interests and safety, stewardship, globalization and social interests. In conclusion, the long-term goal of Health Canada is to protect and promote the health and safety of Canadians by ensuring product identity, purity, potency/strength, bioavailability/delivery and packaging/labeling.

### **Outcomes from Crystal City 2006**

(Dr. Brian P. Booth, Deputy Director, Office of Clinical Pharmacology, Center for Drug Evaluation and Research, US Food and Drug Administration)

The FDA perspective was presented by Dr. Brian Booth. His presentation outlined the highlights from the Crystal City III conference in 2006, and any consequences from the discussions at that conference. In addition to summarizing that information, Dr. Booth expanded on the FDA reaction to each topic. Dr. Booth pointed out FDA concerns that less reliable methods are being used, causing less reliable PK data. In regards to the representative chromatograms submitted as part of NDAs, Dr. Booth mentioned that with respect to NDAs (but not ANDAs) FDA is answering demand and allowing companies to submit fewer chromatograms, however, if more are required at a later date, these should be made available. There is some concern that this approach could cause some delay in review time. Next, Dr. Booth presented the summary slides on the consensus presented at the 2008 AAPS Workshop on GLP Bioanalysis discussing Incurred Sample Reanalysis reproducibility. Discussion of these consensus points can be found further in this paper, in the section "Discussion on Incurred Sample Reanalysis". Finally, it was mentioned that FDA has not started yet, at the time of this Workshop, the process on updating the Method Validation Guidance document. In the meantime, Dr. Booth encouraged the audience to contact representatives in the review division with specific questions. Dr. Booth especially thanked Dr. CT Viswanathan for his help.

Several questions were put forward to Dr. Booth following his presentation, mostly regarding ISR reproducibility. First, it was asked whether or not ISR should be performed on metabolites. Dr. Booth recommended performing the evaluation only on active metabolites. Next, he clarified that with current applications, ISR would only be required retrospectively if the method demonstrated irreproducibility (ISR should be regularly incorporated into all future applications). An attendee questioned if ISR was necessary for urine studies. The response was that if PK data generated were pivotal in nature (e.g. used for labeling the product), then ISR should be evaluated. Dr. Booth was asked how the FDA would interpret a passing evaluation with individual failing samples. He responded that the failing samples should be investigated. Another question was asked about whether it was necessary to perform ISR on both the fast and fed portions of the study. Dr. Booth indicated that in his opinion, only one was sufficient. Finally, it was asked whether a different approach to ISR (documented in an SOP) could be acceptable. The response was that if the approach could be sufficiently justified, and it was documented, then there should be no problem from an Agency point of view.



## Calibration & Validation Group

### **Analytical Investigations**

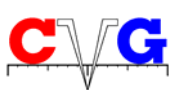
(Michael Lindsay, Director, Bioanalytical Laboratory, Bioanalytical Development, BCD, Apotex Inc.)

Michael Lindsay presented quite thoroughly the topic of analytical investigations. He began by outlining regulatory expectations regarding out of specification (OOS) results. In summary, regulatory agencies expect labs to apply the principles of GLPs to the revision of all results. Guidelines already exist to allow for variation in the results. However, OOS results are possible, and written procedures need to be in place to handle those cases. Investigations need to be timely, unbiased, well documented and scientifically sound. It was clear that although investigations need to be timely, additional data should not be generated until a proper, in-depth investigation is concluded and documented. Only when this initial investigation is not conclusive should a test plan be documented and executed. When the cause is uncovered, corrective actions must be put in place and the potential impact on previously generated data evaluated. Finally, a follow up is necessary in order to confirm that the corrective action successfully solved the problem and the assay is under control.

### **Pharmacokinetic Repeats**

(Dr. Rupinder Phull, Associate Director, Pharmacokinetics & Bioequivalence (PK/BE), Barr Laboratories, Inc.)

Dr. Rupinder Phull began by clarifying the differences between *analytical* repeats and *pharmacokinetic* repeats. Analytical repeats are performed due to a pre-established assignable cause (e.g. instrument malfunction, sample processing error, etc.). Pharmacokinetic repeats are performed on analytically valid results where only the concentration value is questionable. It is mandatory that all repeats be governed by written procedures. The procedure for performing PK repeats presented involved assaying the anomalous value in duplicate, so that three values are available for the sample. An additional approach presented was to assay one sample on either side of the PK repeat as well. Dr. Phull mentioned that acceptance for the duplicate assay should be that there cannot be greater than a 30% deviation between replicates. The final reported value for the sample should be the median of all three available values. However, Dr. Phull, stated that many companies are taking the approach that PK repeats should not be performed. This is due to concerns that doing PK repeats encourages repeat analysis until the desired value is obtained or repeating sensitive samples used in the determination of bioequivalence (e.g. C<sub>max</sub> samples). PK repeats are not recommended for bioequivalence studies. Reasons stated for performing PK repeats include verifying incongruous PK profiles, confirming positive pre-dose levels and suspicions of sample mix-ups. When presenting final results, it is necessary to assess the impact of the PK repeats on the outcome of the study. Data should be presented with and without PK repeat values. PK repeat data should be presented in the analytical report.



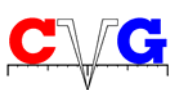
## Calibration & Validation Group

Following the presentation, a comment was brought forth stating that regulatory agencies recommend against performing PK repeats, therefore it is unwise to invest resources in the process. Furthermore, these repeats often indicate GLP issues, which raise different agency concerns, and should be treated as independent of pharmacokinetic analysis. Dr. Phull was asked whether, in her experience, studies where both sets of data were presented (with and without PK repeats), there were any differences noted. She stated that most of the time, there was no significant difference in the presented results. A situation was outlined where the repeat result did not confirm the original. Dr. Phull was asked if the original batch of samples was rejected. Her response was that since additional, non-questionable samples are also assayed and confirm, there is no reason to re-assay the whole run. Finally, as a link between PK repeats and ISR, it was asked if, when an anomalous value was confirmed during the ISR evaluation, a PK repeat would be additionally requested. The answer to this was no, as the result has already been confirmed.

### **Cross Validation of Anticoagulants and Their Impact on the Quantitation of Drugs** (Dr. Saleh Hussain, Director Bioanalytical Operations, Anapharm, A PharmaNet Company)

Dr. Saleh Hussain began by raising two separate issues: different anticoagulants (heparin vs EDTA) and the same anticoagulant with different counter-ions (Na-EDTA vs K<sub>2</sub>-EDTA vs K<sub>3</sub>-EDTA). Regarding the first issue of different anticoagulants, Dr. Hussain noted that at the Crystal City III conference, a consensus was reached whereby there was recognition of the distinct differences of these anticoagulant and therefore there was a need to perform a complete validation of the method. A consensus was not reached, however, regarding the same anticoagulant but different counter-ions. The approach presented was to perform within-run precision and accuracy and long-term stability in matrix. Dr. Hussain presented data of several drugs in K<sub>2</sub>- and K<sub>3</sub>-EDTA, demonstrating that there is no significant difference between the counter-ions. However, he acknowledged that additional testing is necessary and, for the moment, to continue to monitor this conclusion. Furthermore, some agencies have already started asking for test results proving the lack of effects of different counter-ions.

Following this presentation, additional comments were raised by participants. First, it was noted that K<sub>3</sub>-EDTA plasma is approximately 0.4 pH units greater than K<sub>2</sub>-EDTA plasma. Therefore, it would be the pre-processed stability in whole blood that would be most affected by this change. While plasma stability may be an issue it has also been raised that plasma extractions that are susceptible to pH changes (e.g.: SPE extraction) may require consideration and associated validation experiments. Furthermore, it was noted that Na and K are different counter-ions, but that K<sub>2</sub> and K<sub>3</sub> are the same counter-ion with different stoichiometry. Therefore, greater differences could be anticipated between the former pair than the latter pair. Finally, one attendee suggested that when different anticoagulants are used, cross-validation tests should also verify the differences these anticoagulants have in whole blood. Results were observed where there was no



## Calibration & Validation Group

effect on the method in plasma. However, the anticoagulant affected the partitioning of the analyte between the blood cells and the plasma.

### Criteria for Selecting PK Repeat Study Samples

(Dr. S. Peter King, VP, Global DMPK, Quest Pharmaceutical Services (QPS), L.L.C.)

Two points of view were presented: Pharma and CRO. The Pharma point of view is that when a run meets bioanalytical acceptance criteria, all data generated as part of that run should be acceptable. Exceptions to this rule could be if a data point in the elimination phase of the profile shows a second peak that is inconsistent with other subject profiles or if pre-dose or placebo samples demonstrate detectable analyte concentrations.

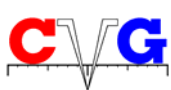
The CRO point of view is that an SOP is required to dictate the procedure and criteria for selecting PK repeats. However, PK repeats should not be performed for bioequivalence studies unless specifically requested by the sponsor. In this case, sufficient justification should be provided in writing by the sponsor. In cases where PK repeats are performed, several questions should be asked regarding inter- and intra-subject variability and the consistency of the time point in relation to other similar time points and subjects.

Following the presentation, many points were raised by the attendees. First, the CRO has no stake in performing PK repeats. However, for a sponsor the impact can be major. Second, if performing a PK repeat for the parent analyte and not the metabolite, additional metabolite data is now available, and there is no consensus on what to do with that extra data. Furthermore, it is unclear what constitutes “sufficient justification” by a sponsor in order to perform PK repeats for a bioequivalence study. Dr. King responded that in his opinion, there is very little justification available, and therefore they should not be done. Finally, one company performed a retrospective audit of all their data where PK repeats were performed, and found that in ~99% of the cases the PK repeats had no significant impact on AUC and C<sub>max</sub>.

### What Changed after Crystal City III: A CRO Perspective

(Dr. Nicola C. Hughes, Director Bioanalytical Laboratory Biovail Contract Research, A Division of Biovail Corporation)

Following the Crystal City III conference, where several issues were discussed, many minor and major changes were proposed that have an effect on CROs. Dr. Hughes addressed the reactions to these changes from a CRO perspective. In summary, those changes that were easily understood and easily implemented were: including 2 QC levels within the concentration profiles, assessing contamination and carryover during routine sample analysis and not only during validation, reporting data and reasons for failed batches, performing stability evaluations against *freshly* prepared standard curves or stocks, and removing system suitability tests as a requirement (now a recommendation only). Those changes that required more time, understanding and discussion were the subjects of incurred sample reanalysis and matrix effect testing.



## Calibration & Validation Group

### **Challenges & Strategies in Bio-Analytical Method Development**

(Zhimeng Zhu, Director, Biopharmaceutics & Clinical Research Department, Pharmascience Inc.)

Ms. Zhu presented a case study to illustrate strategies for developing a robust method. Her case demonstrated that an unknown metabolite converted back to the parent compound under basic extraction conditions. Additionally, the use of methanol also caused inter-conversion. Tests during development determined that a protein precipitation extraction with ACN was the solution to this difficult case. Therefore, strategies to adopt during method development are an initial, thorough literature search, use of a stable-labeled internal standard, a chemistry driven pre-validation plan, cross-validation of different liquid chromatography columns, analysts and systems during pre-validation and comprehensive employee training on the method.

### **Comparison of Variation Between Replicated Incurred Samples and QC Samples**

(Eric Ormsby, A. Manager, Office of Science, Health Canada - Therapeutic Product Directorate)

Following the concerns raised during the 1<sup>st</sup> CVG Workshop in 2007 regarding the possibility of large discrepancies between replicates during the ISR evaluation, it was noted that Health Canada had an archive of information available from the 15% replicates data that was submitted prior to 2003. Therefore, a call went out for volunteers to submit past replicate data, along with applicable QC results. All data was submitted anonymously. Only drug name, extraction method and detection method information was included.

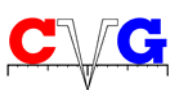
35 assays were submitted both in plasma and urine. Results demonstrated that variation in incurred samples was larger than the variation in QC samples. Furthermore, one statistical analysis demonstrated some outlier results that were difficult to interpret due to the small sample size of the available data. However, Dr. Ormsby was clear that these results are just preliminary, and additional data are needed in order to obtain a more representative sample size. Therefore, he asked that anyone interested in supplying more data should do so to the following address:

[http://www.hc-sc.gc.ca/dhp-mps/prodpharma/activit/annonce-annonce/notice\\_bioan\\_avis\\_anbio\\_e.html](http://www.hc-sc.gc.ca/dhp-mps/prodpharma/activit/annonce-annonce/notice_bioan_avis_anbio_e.html)

In conclusion, Dr. Ormsby stated that Health Canada regulatory requirements would not change until there is a better understanding of the need.

### **Drug Biotransformation in Incurred Samples: Impact of Acyl Glucuronide and N-Oxide Metabolites during Sample Processing**

(Dr. Fabio Garofolo, VP Bioanalytical Services, Algorithme Pharma Inc.)



## Calibration & Validation Group

Dr. Garofolo discussed the impact of biotransformations occurring during sample processing. These metabolic conversions typically occur during sample extraction or during system analysis. Several case studies were presented to illustrate the challenge these conversions can cause during method development, and some solutions that can be applied to overcome these challenges.

During sample analysis, examples of possible conversions are the hydrolysis of acyl glucuronides, the reduction of N-oxides and the opening or closing of lactones. These conversions can be caused by instability in room temperature or during evaporation, suboptimal pH during extraction, or the use of reactive solvents. Some possible solutions presented involved thawing samples in an ice bath, adding preservatives, or using inert solvents.

During instrumental analysis, conversion can arise from a degradation of Phase II metabolites or N-oxide occurring in the source or on the column due to the chosen mobile phase. These challenges can be overcome by avoiding APCI if possible, choosing a non-reactive mobile phase, or having labile metabolites chromatographically separated.

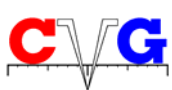
Dr. Garofolo went on to conclude that incurred sample reanalysis is useful to evaluate reproducibility of metabolites prone to degradation. The accuracy of the method can be significantly affected by the presence of acyl glucuronides and N-oxide metabolites. Furthermore, acyl glucuronides behave differently under different conditions, and do not necessarily degrade into the parent compound. It is recommended to test the metabolites during all phases of method use (development, production and ISR) and to use synthesized labile metabolites whenever possible to monitor their effects during use of the method.

### **PANEL DISCUSSION**

The second day of the workshop involved discussion on the various topics addressed in the presentations, and proposed by the attendees and CVG members (listed in the Goals and Objectives section of this paper). Outlined below is a summary of those discussions, as well as any consensus reached by the panel on any of the topics.

### **Discussion on Matrix Effects and Hemolysis Effects**

**Topic introduction:** Matrix effect occurs when matrix ions co-elute with the analyte(s) of interest and influence ionization such that there might be a suppression or enhancement of peak response, and hence, a significant effect on quantitation. One special case of a matrix effect is the hemolysis effect. It is commonly accepted that as long as the matrix effect is consistent, then its presence is acceptable. One approach to determining matrix effect is calculating the matrix factor, another is quantifying the analyte in different matrix sources. However, each approach is not without their limitations.



## Calibration & Validation Group

**Panel Consensus:** Matrix effects and hemolysis effects should be considered since they can have a significant impact on the ruggedness of the method. The evaluation may be performed as part of method development or method validation. However, regardless of the location of the results, the test needs to stand up to a regulatory audit.

**Issues raised during discussion:** Although there was general agreement by the attendees toward the consensus, it was unclear how the level of hemolysis is quantified and therefore how its effect is evaluated. In general, those who perform the test use a significantly hemolysed sample which they have defined as 2% (prepared in-house by adding 2% whole blood to plasma). Furthermore, there was a suggestion that lipemia tests will be required as well in the future as it is a current requirement for the ANVISA.

### Discussion on Ion Suppression and Matrix Effects

**Topic introduction:** Furthering the discussion above, an additional approach to determining the effects of matrix on ionization suppression or enhancement is the post column infusion. Matrix effects can be different depending on the species being tested.

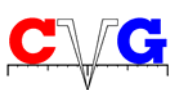
**Panel Consensus:** When the same compound is being tested in different species, it was generally agreed that a full validation should be performed for each species. Furthermore, to perform the matrix effect test, the matrix factor can be used as an evaluative tool. However, it was generally agreed that it is not representative of sample analysis. An alternate approach is to spike six different lots of matrix and determine their back-calculated concentrations.

**Issues raised during discussion:** A question was put forth to the panel asking what criteria they set for internal standard response of the standards/QCs versus subjects. Some respondents indicated that the IS response in subjects should be between 50% and 180% of the mean of the curve. Different criteria may be applied if the IS is stable-labeled since there is an assumption that a stable-labeled internal standard behaves exactly as the analyte of interest. Additionally, some apply an internal standard outlier test. An avenue of investigation in this situation may be to spike pre-dose samples of the subject in question as a QC sample and calculate the back-calculated concentration in order to determine the reliability of the data from that subject.

### Discussion on Autosampler Stability and Re-Injection Reproducibility

**Topic introduction:** Following the Crystal City III conference, clarification regarding the extract stability and autosampler re-injection reproducibility was outlined. If samples are stored prior to placement on an instrument, then that stability needs to be proven, typically against a freshly prepared standard curve. However, a freshly prepared standard curve is not deemed necessary for autosampler reinjection reproducibility.

**Panel Consensus:** It was clear during the consensus discussions that the objectives of both evaluations are very different and should be taken into consideration. Performing



## Calibration & Validation Group

both evaluations requires extra work. However, each approach gleans different information. The re-injection reproducibility is performed by freshly preparing standards and QCs, injecting, storing under autosampler conditions and then re-injecting. Autosampler stability ensures that there is stability between the beginning and end of the batch.

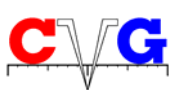
**Issues raised during discussion:** It was clarified during discussions that the autosampler stability should be run against freshly prepared standards and QC samples, although it is acknowledged that a different preparation of a standard curve introduces inter assay variability. Additionally, due to practical considerations, it is necessary to prove that a batch may be re-injected (i.e. due to system interruptions or other analytical reasons). Attendees stated that 483s are being issued if autosampler stability is not being done. Therefore typically both evaluations are being performed.

Moreover, there was some discussion about the multiple elements that are under the rubric of “Post Preparative Stability”:

1. Processed Sample Stability – This answers the question: Can I take a set of samples, processed as a batch, store them for  $n$  hours, and then inject them as a single batch run? (Note: not a re-injection, but an initial injection). A fresh calibration curve should not be required for this experiment.
2. Autosampler Stability – The aim of this experiment is to demonstrate that the samples are stable over the time interval required to analyze a full study batch run. In a typical study run, unknowns injected near the end of the batch are back-calculated against calibrators injected at the beginning of the batch (similar considerations would apply even if samples are run in a randomized order or if unknowns are bracketed by two calibration curves). A fresh calibration curve should be used for this experiment.
3. Re-injection reproducibility – This answers a separate question: Can samples be re-injected with acceptable accuracy/precision? The critical variable with re-injections is that the sample closure has been pierced, potentially resulting in evaporation, changes in solubility, sample oxidation, etc. Does this experiment need to be run with a fresh calibration curve? If the lab’s practice during study sample analysis is to re-inject individual study samples and back-calculate them against the initial injection of calibrators, then yes (this would be similar to Autosampler Stability above). If, however, the lab’s practice is to re-inject entire batches (i.e. calibrators, unknowns and QCs), then this is a case analogous to Processed Sample Stability and a fresh curve would be unnecessary.

### **Discussion on 483s**

**Topic introduction:** Due to the fast changing nature of the industry, and the inability of regulatory agencies to update the guidances at the same rate, the prevalent view is that standards are being set by 483s, and not by consensus. Pharma and CRO companies are used to taking in serious consideration the 483s issued to others in the industry, and may, in response, overreact by changing procedures prematurely.



## Calibration & Validation Group

**Panel Consensus:** The reality of the situation is that Regulatory Agency standards are initially communicated verbally (e.g.: ISR) at conferences and workshops and then being enforced by 483s. This results in the current climate. Since regulations and guidances cannot keep up with the science in all cases, this is the method that agencies have found to set the standards for the future. Using the opinions and consensus points reached at meetings such as the CVG, BSAT-APA and AAPS Workshops, the FDA uses 483s to enforce a common understanding of a standard. A suggestion was presented to the attendees for having regular meetings in North America on Regulated Bioanalysis throughout the year where Regulatory Agencies, Pharmas and CROs can openly discuss the major common concerns and hot topics so that there is continuous industry consensus on how the issue will be approached. The panelists and the audience agreed on the importance of these workshops. CVG (Dr. Garofolo) and AAPS Bioanalytical Focus Group (Dr. Stephen Lowes) representatives committed to further discuss the possibility to collaborate on regular open forums for discussion.

**Issues raised during discussion:** Dr. Viau from Health Canada encouraged the development of a task force, and stated that Health Canada would participate if invited to join. Currently, Health Canada produces “Notices to Stakeholders” in order to publish the agency’s current views. The majority of attendees were in favor of forming the task force.

### **Discussion on Storage Temperature (-70°C versus -80°C)**

**Topic introduction:** There is an uncertainty within the industry as to whether -70°C and -80°C are equivalent. Furthermore, discussion at the Crystal City III conference centered on the question of stability performed at -20°C being valid at -80°C.

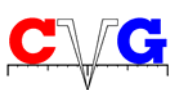
**Panel Consensus:** A consensus was reached stating that -70°C and -80°C were equivalent.

**Issues raised during discussion:** It was clarified during the discussion that if the set point of the freezer is  $-80^{\circ}\text{C} \pm 10^{\circ}\text{C}$ , then this is the reported stability temperature (as opposed to reporting -70°C because it is the warmest temperature in the range). The majority of the attendees agreed with this position, and they are currently functioning this way. They are typically using storage temperatures of -80°C. However, no consensus was reached on the subject of a stability performed at -20°C being valid at -80°C.

### **Discussion on Anticoagulant Counter Ions**

**Topic introduction:** Currently, several labs are receiving non-compliance letters from the TPD requesting cross-validation when changing anticoagulant counter ions. This is not the approach used by other Agencies. Therefore, there is confusion within the industry as to the reasons behind the request, as well as what evaluations are needed.

**Panel Consensus:** There was no consensus within the panel.



## Calibration & Validation Group

**Issues raised during discussion:** The Health Canada representatives had no details regarding these non-compliance letters. However, it was made clear that although there seems to be no impact on the methods when using a different counter ion, the responsibility is on the lab to prove it. No consensus was reached on what cross-validation evaluations are necessary. However, in one case, the non-compliance letter requested the performance of the long-term stability in matrix.

### **Discussion on the Acceptance of Non-Linear Calibration Models**

**Topic introduction:** Regulatory agencies do not recommend the use of quadratic calibration models unless absolutely necessary. However, this raises the question of how much quadratic is too much.

**Panel Consensus:** The general consensus by the panel was that it should be avoided. However, if a least squares approach demonstrates that it is the best fit, it can be used as a last resort.

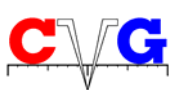
**Issues raised during discussion:** Most of the panelists do not use quadratic at all. However, it was noted that it was used more frequently in ligand binding assays and for broad dynamic ranges. The position of Health Canada was that use of quadratic calibration models is acceptable if it is used for all curves. If the model fluctuates between linear and quadratic, then the ruggedness of the method will be questioned. One proposal was to add additional high calibrants to get a better sense of at which point in the curve the quadratic tendency begins. It was also pointed out that in ligand binding assays, where quadratic models are used, anchor points are defined to limit the maximum and minimum usable points on the curve.

### **Discussion on Contamination Criteria**

**Topic introduction:** Contamination of blank samples, regardless of the cause, needs to be monitored during sample analysis and method validation. Acceptance criteria are outlined in the each agency's guidelines and regulatory documents.

**Panel Consensus:** A criteria of <20% of the lower limit of quantitation (LLOQ) is used overwhelmingly in the industry.

**Issues raised during discussion:** One alternative criteria was presented where the company used <33% of the LLOQ. This is due to the fact that this company has additional criteria where the signal-to-noise ratio (SNR) for the LLOQ must be at least 10. Additionally, any peak that is integrated must have a SNR of at least 3.



## Calibration & Validation Group

### **Discussion on Pharmacokinetic Repeats**

**Topic introduction:** Discussions are ongoing in the industry on the validity of performing pharmacokinetic (PK) repeats and the criteria used for selecting them. These discussions overlap with discussions of laboratory investigations in that PK repeats often bring to light analytical errors that should be corrected prior to submitting the data to PK. Additionally, there is some question on the impact of PK repeats on data submitted to the agencies.

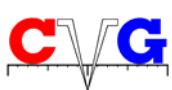
**Panel Consensus:** No consensus was reached by the panel. However, it was highlighted that investigations need to be performed when anomalous results are obtained. This investigation should be performed prior to generating additional data, however, if repeated data is needed, it can be generated under the umbrella of the investigation as long as criteria for reporting the data is documented prior to reanalysis.

**Issues raised during discussion:** This topic was discussed at length, and many opinions and comments were offered. First, if PK repeats are performed, and the result does not confirm, an investigation into the original batch should be triggered. Another question raised was whether the criteria for selecting PK repeats should also be applied to outlier QC samples so that an investigation is triggered as well. This point of view is based on the fact that failing QC samples are not investigated the way unknown samples are, therefore results are not treated objectively. Therefore, it was generally agreed that anomalous results should be investigated, and a criteria for opening an investigation needs to be outlined in an SOP. Analytical investigations should be performed prior to submitting data to PK, so that only analytically sound data are presented. However, it was stated that it is not necessary to investigate each point in the profile, and that the concern becomes objectively evaluating sensitive time points (e.g. C<sub>max</sub>). Only suspicion of actual analytical problems should be investigated (e.g. sample switching, carry over, etc.). There is a fine line between analytical repeats and PK repeats. If PK repeats are performed, it is essential that an SOP is written detailing the justification for the procedure, the criteria for selecting these samples and the method of selecting the reported value. Finally, the TPD is in the process of preparing a guidance document on the subject (proposed release in spring 2009).

### **Discussion on Incurred Sample Reanalysis (ISR)**

**Topic Introduction:** Following the Crystal City III conference, general acceptance of this evaluation has occurred throughout the industry. Several companies have drafted a procedure based on the guidelines presented in the corresponding White Paper. Additionally, the AAPS held the Workshop on GLP Bioanalysis Discussing Incurred Sample Reproducibility on February 7-8, 2008 in Arlington, VA, USA. At this workshop, the steering committee reached a consensus on the following 14 points:

1. ISR reinforces confidence that the method is valid and reproducible.
2. An SOP must be in place to govern ISR

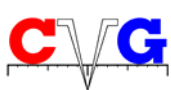


## Calibration & Validation Group

3. ISR must be performed if PK data is the primary objective of the study
4. Individual samples must be used, not pooled samples (exceptions can be made for studies with limited volumes, special patients, etc.)
5. ISR should be conducted early in the study
6. ISR should be conducted using the same number of replicates as used to run the study
7. ISR data should be presented in the analytical report
8. ISR reproducibility failure does not always mean study rejection (some objectives of the study can still be met)
9. For pre-clinical studies, conduct ISR once for each species/method/lab
10. For pre-clinical studies, ISR constitutes part of validation activities
11. For clinical studies, ISR is performed for BA/BE studies, healthy volunteer studies, patient studies, and drug-drug interaction studies
12. When choosing samples, approximately 20 samples should be chosen. More subjects with fewer sampling times is preferred to using full subjects (one sample near the C<sub>max</sub> and one near elimination phase)
13. A failed ISR reproducibility evaluation must lead to an investigation
14. The acceptance criteria recommended is 2/3 of replicates within 20% of the original value

**Panel Consensus:** The panel agreed with the procedure outlined in the Crystal City III White Paper, as well as with the consensus points presented at the AAPS Workshop. There was some question as to the acceptance criteria presented at the AAPS workshop, however, the panel at the CVG uses either 20% difference from the original value or within 20% of the mean. Health Canada is currently reviewing this issue but maintains the position that IRS should be part of method validation and determined before the bio study is initiated.

**Issues raised during discussion:** Generally, all attendees had either successfully implemented the evaluation or were in the process of drafting the procedure. The approaches used for the procedure were generally in agreement with the proposals outlined in the Crystal City III White Paper and the AAPS Workshop. The acceptance criteria most widely used was 2/3 of replicates within 20% of the mean of the values.



## Calibration & Validation Group

### **ACKNOWLEDGMENTS**

On Behalf of CVG, Dr. Garofolo would like to thank:

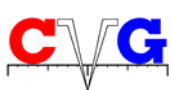
- FDA and TPD for supporting this Workshop
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### **THE 3<sup>rd</sup> WORKSHOP ON RECENT ISSUES IN GLP BIOANALYSIS**

The 3<sup>rd</sup> Workshop on Recent Issues in GLP Bioanalysis will be held on April 16-17, 2009 in Montreal, Quebec, Canada.



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### **ACRONYMS**

AAPS: American Association of Pharmaceutical Scientists  
ANVISA: Agência Nacional de Vigilância Sanitária (National Health Surveillance Agency Brazil)  
AUC: Area Under the Curve  
BA/BE: Bioavailability/ Bioequivalence  
BSAT-APA: Boston Society for Advanced Therapeutics - Applied Pharmaceutical Analysis  
Cmax: Maximum Plasma Concentration  
CVG: Calibration & Validation Group  
EDTA: Ethylenediaminetetraacetic Acid  
FDA: Food & Drug Administration  
GCP: Good Clinical Practices  
GLP: Good Laboratory Practices  
GMP: Good Manufacturing Practices  
ISR: Incurred Sample Reanalysis  
LLOQ: Lower Limit of Quantitation  
OOS: Out of Specification  
SOP: Standard Operating Procedure  
SPE: Solid Phase Extraction  
PK: Pharmacokinetic  
QC samples: Quality control samples  
TPD: Therapeutic Product Directorate

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