Outcomes of the Global Bioanalysis Consortium’s Recommendations: Small Molecule and X-Modality Discussion Topics

Eric Fluhler on behalf of the SM and Mixed Topic Harmonization Teams
S1 – Specific Run Acceptance

• Dilution QC’s – Sample Analysis
  – Dilution QC’s are only used for acceptability of samples analyzed after dilution
  – Dilution QC’s only need to be run at the maximum dilution level/factor for that run

• Reinjection reproducibility/autosampler stability – Validation
  – Full run reinjected during validation
  – If reinjected QC’s pass against original curve, a partial batch can be reinjected during sample analysis

Req QC’s?
S1 – Specific Run Acceptance

• Internal Standard Testing
  – IS reproducibility requirement should be defined in SOP or method, *a priori*

• Selectivity and Carryover
  – Signal in blanks ≤ 20% LLOQ signal for selectivity and carryover preferred criteria
S2 – Specific Assay Operation

• Selectivity
  – 6 Lots; 1 Hemolyzed (2% WB in plasma); 1 Hyperlipidemic (HL), clinical only (spiked Intralipid IV @ 20 mg/mL)
  – Assess as lack of impact on LLOQ

• Carryover
  – Assess in each run. Failure causes impact assessment rather than outright batch rejection
  – Assess using LLOQ after high standard
  – Acceptance: fixed % or based on P/A of assay
S2 – Specific Assay Operation

• System Equilibration
  – Document in method and study file
  – No unanalyzed study samples, Stds, QC’s
  – Not an absolute requirement for assay

S3 – Chromatographic Run Quality

• An SOP on IS response should be in place, but does not need to be prescriptive (i.e. mean +/- 50%, or similar)
A2 – Tiered Approach for MV

• Practicality in 483 averse environment
• 4 tiers proposed (screening, research, qualified, validated)
• When to apply each, which are auditable
A3 – Method Transfer and Cross Validation

• Transfer is a process **not** a cross validation
  – Internal: Two P/A batches (4 for LBA)
  – External: Full validation & inter-lab comparison ("may" included spiked QC’s)

• Cross validation consists of analysis of assessment samples (spiked QCs and incurred samples) assayed using two or more different validated methods
A4 – Reference Standards and Reagents

• Calibrators and QC samples should be prepared from a **verified** stock solution with proven stability

• LM: New reference standard qualification
  – Qualified via single partial validation run with L, M, H validation QC samples (n=6)

Alternate: Stds and QC’s from each lot with QC’s read from each curve.
A6 – Stability

• Stability in WB not required
• Stability in the presence of co-meds or in unique matrix (e.g. hemolytic) not required
• Incurred sample stability not required

Unless Scientifically Called For
A9 – Instrument Qualification

• Operations must lead effort and be guided by internal SOPs. It is not appropriate for manufacturer to do it all.

• **System suitability is required** in some form for LC/MS, Immunoassay and any other Bioanalytical techniques.

• Laboratories should have a **requalification process** in place to address instrument issues and disruptions.

• Both activities must be defined in an SOP.

• AIQ based on GMP is inappropriate. BA runs have qualification built in via stds, QCs, etc.
  – Testing instruments for Regulated Bioanalysis should be application specific
  – Holistic, application specific PQ testing is one way to achieve this
A7 – Reanalysis and ISR

• Incongruous result repeats
  – Select prior to PK analysis, repeat in duplicate
  – Accept if reassay if difference \( \leq 30\% \); \( \leq 40\% \) for LBA
    (Feedback needed, align with ISR %?)
  – Report median of 3 results
A7 – Reanalysis and ISR

• ISR
  – Largely a QC effort, scientific return obtained after first test per unique matrix

• Amount of ISR
  – 5% of samples for all studies (eliminate tiers)
  – Minimum of 20 samples

• ISR Failures
  – How to deal with individual outliers
A7 – Reanalysis and ISR

• ISR Selection
  – Based on visual inspection, low/high concentration range of samples, > 3XLLOQ

• ISR for Multi-analyte methods
  – Select based on primary active entity
  – Consideration to other analytes, but not driven by them unless they represent a major metabolite or co-med and are largely separated pharmacokinetically
Discussion Topics

- Dilution QC’s
- Reinjection reproducibility/stability
- IS reproducibility
- Selectivity/Carryover (% of LLOQ vs. LLOQ perf.
- System Equilibration vs System Suitability
- Tiered MV
- Method Transfer/Cross Val
- Ref Std prep and qualif.
- Stability: ISS, WB, co-meds

- System suitability and requalification
- Repeat analysis for incongruous results
- ISR amount, selection and individual sample failures
Backups
S1 – Specific Run Acceptance

- **Dilution QC’s – Validation**
  - Validate a single dilution concentration at a single dilution factor (e.g. 10X) at highest concentration expected in study samples

- **Dilution QC’s – Sample Analysis**
  - Include dilution QC’s in sample analysis run only if dilutions are made
    - Dilution QC’s are only used to evaluate the acceptability of samples analyzed after dilution
    - Dilution QC’s only need to be run at the maximum dilution level/factor for that run
S1 – Specific Run Acceptance

• Sample and Run Reinjection
  – For reinjection reproducibility/autosampler stability, a complete validation batch is reinjected
  – Reinjected QC’s are compared separately to both original and reinjected calibration curves

  ❖ If reinjected batch passes, full batches can be reinjected during sample analysis
  ❖ If reinjected QC’s pass against original curve, a partial batch can be reinjected during sample analysis
S1 – Specific Run Acceptance

• Selectivity and Carryover
  – Use blank matrix to test carryover, selectivity, recovery, matrix effects, etc.
  – Signal in blanks ≤ 20% LLOQ signal for selectivity and carryover preferred criteria
S1 – Specific Run Acceptance

- Internal Standard Testing
  - Certificate of Analysis **not** required
  - Long term stability testing **not** required
  - **Demonstrate** IS does not interfere
  - IS reproducibility requirement should be defined in SOP or method
    - **Objective criteria should be established apriori to address situation**
S2 – Specific Assay Operation

• System Equilibration
  – Document in method/procedure
  – Never use unanalyzed study samples
  – Do not use a sample that could be substituted in actual run (e.g. std1, LQC,..)
  – **Not** an absolute requirement for assay
  – All injections made prior to injection of a batch should be documented in study file
S2 – Specific Assay Operation

• Selectivity
  – 6 Lots
  – 1 Hemolyzed (2% WB in plasma)
  – 1 Hyperlipidemic (HL) ; clinical only (spiked Intralipid IV @ 20 mg/mL)
  – Assess as lack of impact on LLOQ

• Matrix Effect (2M, 2F, 1Hemo, 1 HL)
  – Base on IS normalized results
  – Low and high concentrations (CV ≤ 15%)
S2 – Specific Assay Operation

• Carryover
  – Assess in each run. Failure causes impact assessment rather than outright batch rejection
  – Assess using LLOQ after high standard
  – Acceptance: fixed % or based on P/A of assay
S3 – Chromatographic Run Quality Assessment

• Integration
  – Integration parameters used in assay validation should be considered as a starting position for run integration. **It is scientifically invalid to assume that chromatographic integration parameters must be fixed.**
  – Integration of chromatographic peaks should be consistent for calibration, QC and incurred samples within a run
  – Initial integration: process that occurs **prior** to regression; automated, modified automated and manual.
  – Reintegration: process that occurs **post** regression following peer reviewed rejection of initial integration
S3 – Chromatographic Run Quality Assessment

• An SOP describing IS response should be in place, but this does not necessarily need to have restrictive mathematical criteria (i.e. mean +/- 50%, or similar)

• Peer review of a batch (post regression) should include 100% audit of chromatograms for consistency of both chromatography and integration
A1 – Scope and Regulations

• Defined and contrasted Regulations, Guidance and White papers

• Regardless of GxP, recommendations made for performing Regulated BA with an hierarchical structure
  – Validations to follow regulated BA principles
  – Samples analysis following regulated BA principles, while maintaining GLP and GCP status for samples

• Recommended a draft scope statement for GBC whitepaper
  – Clarified scope for regulated bioanalysis
  – Included timelines for validations and scope (extent) of validation before analysis of samples

• Created draft glossary and abbreviations for BA
A2 – Tiered Approach for MV

- Practicality in 483 averse environment
- 4 tiers proposed (screening, research, qualified, validated)
- When is each tier applied?
A2 – Tiered Approach for MV

Screening, research or qualified methods as desired

Parent drug & post-MIST* metabolite in plasma: validated method
Parent drug & post-MIST metabolite in other matrices: qualified method

Discovery

Non-GLP Preclinical or in vitro

IND GLP tox

Ph 1 or 2 & Chronic GLP tox

Ph 0

Ph 1

Ph 2

Ph 3

Post approval

Parent drug in plasma: validated method
Parent drug in other matrices: qualified method
Metabolite/Prodrug in any matrix: qualified method
Human vs. animal plasma for MIST: screening method

Parent drug, prodrug or metabolite in any matrix: qualified method
A3 – Method Transfer and Cross Validation

• Transfer is a process not a cross validation
  – Internal: Two P/A batches (4 for LBA)
  – External: Full validation & inter-lab comparison ("may" included spiked QC’s)

• Matrix stability not repeated in cross/partial validations

• Cross validation consists of analysis of assessment samples (spiked QC’s and incurred samples) assayed using two or more different validated methods
A4 – Reference Standards and Reagents

• Calibrators and QC samples should be prepared from a **verified** stock solution with proven **stability**

• Altered or surrogate matrix should only be used if equivalency is demonstrated between surrogate matrix and authentic matrix. QC samples should be prepared in authentic matrix
A4 – Reference Standards and Reagents

• LM: New reference standard qualification
  – Qualified via single partial validation run with L, M, H validation QC samples (n=6)

Alternate: Stds and QC’s from each lot with QC’s read from each curve.
A5 – Sample Management
A6 – Stability

- Stability in WB **not required** unless scientifically called for; assumes plasma/serum stability under the same conditions.
- Stability in the presence of co-medications or in unique matrix (e.g. hemolytic) **not required** unless scientifically called for.
A6 – Stability

• Use of freshly prepared calibrators recommended for long-term stability only

• Incurred sample stability not required as a standard. It should serve to bridge a possible gap between spiked and incurred samples, when deemed necessary based on the physicochemical and/or metabolic properties of the analyte.
A7 – Reanalysis and ISR

• Incongruous repeats
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A8 – Documentation

• High level table of contents proposed for the analytical and validation report
• Recommendation to reserve a separate section in CTD on bioanalysis to avoid confusion and help reviewers locating the data.
A9 – Instrument Qualification

- Operations must lead effort and be guided by internal SOPs. It is not appropriate for manufacturer to do it all.
- **System suitability is required** in some form for LC/MS, Immunoassay and any other Bioanalytical techniques.
- Laboratories should have a **requalification process** in place to address instrument issues and disruptions.
- Both activities must be defined in an SOP.
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  - Testing instruments for Regulated Bioanalysis should be application specific
  - Holistic, application specific PQ testing is one way to achieve this
A10 – New Frontiers
A11 – Biomarkers