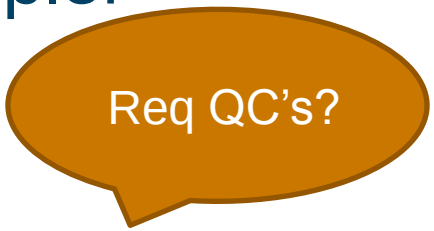
Outcomes of the Global Bioanalysis Consortium's Recommendations: Small Molecule and X-Modality Discussion Topics

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



S1 – Specific Run Acceptance

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

Criteria?

- 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 out right 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

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 ≤ 30% ; ≤ 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 reprod/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 out right 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

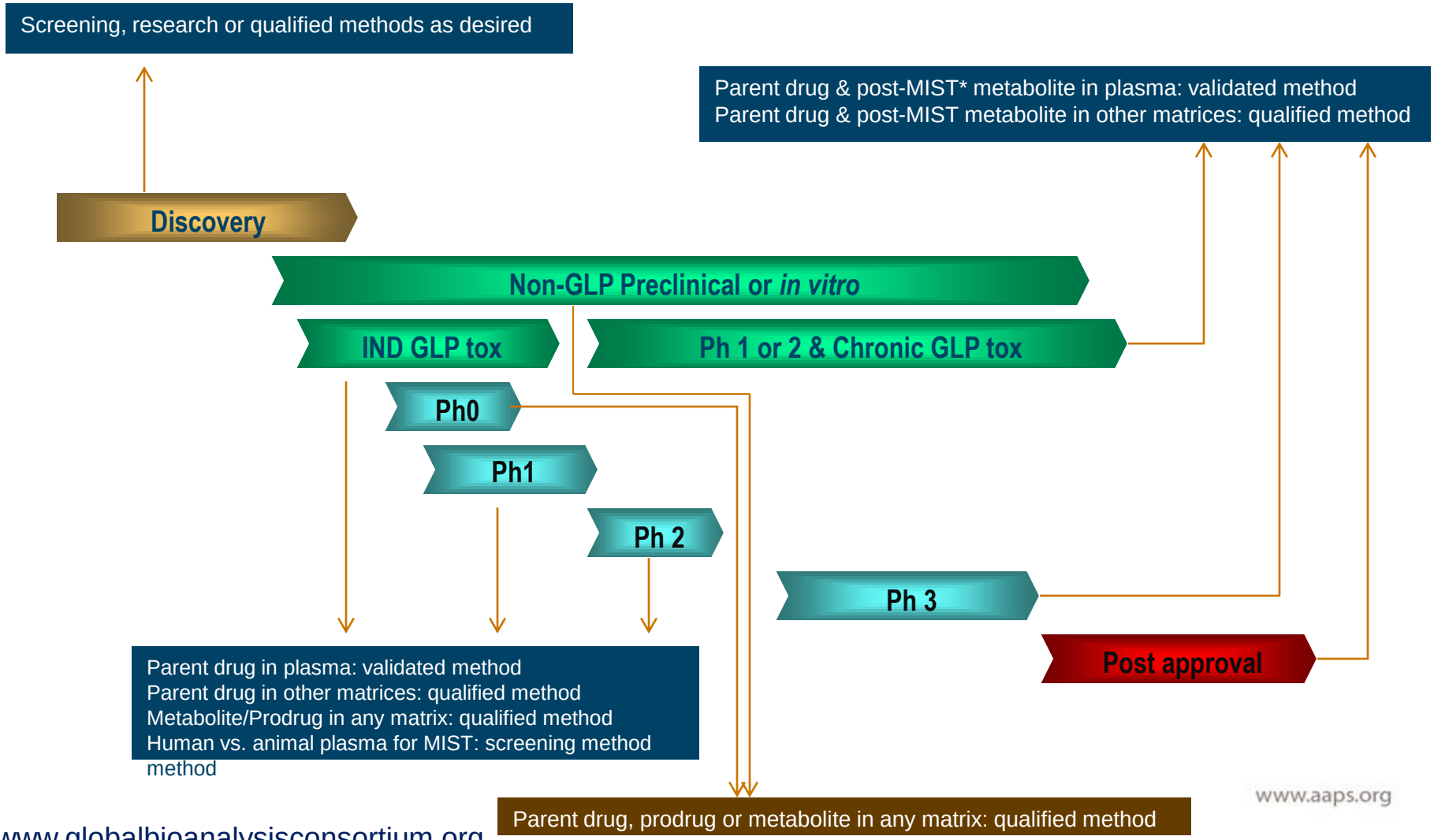
A1 – Scope and Regulations

- 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



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 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
- 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-medicatiions 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
  - Select prior to PK analysis
  - Repeat in duplicate
  - Accept if reassay if difference ≤ 30% ; ≤ 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
  - 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

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.
- 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

A10 – New Frontiers

A11 – Biomarkers