GLOBAL BIOANALYSIS CONSORTIUM

Team S2 Update for the EBF Focus Meeting

Steve White for the S2 Team

13 June 2012

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Scope of Discussions

- Results to be Presented Today
  - System Equilibration
  - Selectivity with respect to Hemolyzed and Lipemic Samples
  - Carryover and contamination
  - Matrix Effects

- Additional Topics on Which Consensus was Reached
  - Sensitivity
  - Specificity/Selectivity
  - Recovery
  - Preparation of Calibrators

- Topics Discussed by Other Teams on Which Consensus was Reached
  - Sample Reinjections/Anomalous Results/Failed Runs (Team S1 will Present)
  - Impact of Salt/Counter Ion Changes (Team S1 will Present)
  - Internal Standard Variability (Team S3 will Present)

System Equilibration
(System Suitability covered by S1 Team)

- Never use unanalyzed test samples
- Do not use a sample that could be substituted in official run (std 1, low qc, etc.)
- Use pooled samples, yesterdays samples, purpose generated (should not be at assay std or qc conc.)
- Assess adequate equilibration through precision of sequential injections of the test sample.
  - Precision should be stated in assay protocol
- Not an absolute requirement for an assay
- All injections made prior to injection of a batch need to be documented in the study file

Selectivity
(Hemolyzed and Lipemic Samples)

- Analytical method should be able to differentiate the analyte(s) of interest and IS from endogenous components in the matrix or other components in the sample.
- Selectivity should be proved using at least 6 individual sources of the appropriate blank matrix, which are individually analyzed and evaluated for interference.
  - **Include 1 hemolyzed lot.**
  - Generally a hyperlipidemic lot should be included only for clinical assays unless a special population of animals is employed.
  - What happens if one lot fails
    - start with 6 lots, if a lot fails, increase to 10 lots and then 90% need to meet acceptance
- Definition of lack of interference
  - 20% analyte LLOQ/5% istd? How to calculate?
    - lack of impact in quantitation at LLOQ. As part of validation run LLOQ in multiple lots.
- Hemolyzed/hyperlipidemic definitions
  - Hemolyzed matrix: 2% whole blood in plasma.
  - Hyperlipidemic matrix: control plasma to which a commercial lipid product; Intralipid IV (available from Sigma-Aldrich), is added to give a lipid level (20 mg/mL) which is10x borderline high
  - Multiple degrees? – no, work at extremes
  - Internal standard response should be monitored as a diagnostic during sample analysis

Carryover and Contamination

- Carryover vs. contamination
  - carryover is a response caused by injection process and is variable during run (depending on previous sample)
  - contamination is a response caused by other factors (sample collection and processing) and may be in the sample when received at the BA lab
- Carryover needs to be understood and minimized during assay development
  - assay range may need to be adjusted to minimize carryover impact
- Need to differentiate contamination from endogenous in the case of a naturally occurring analyte
- Methodology to assess carryover
  - injecting blank after high standard may give a response that cannot be easily integrated, especially if LLOQ signal is already at s/n=5
  - injection of an LLOQ sample after a high standard is a better approach to assess carryover impact
    - acceptance criteria may be either a fixed %difference of response or a variable difference based on the precision of the assay as assessed during validation **- input sought**

Carryover and Contamination (cont.)

- Carryover should be assessed during each run, minimally twice, once at the beginning and once at the end of the run
  - carryover test failure causes impact assessment rather than out right run rejection
  - use average LLOQ response if duplicate std curves run
- Should there be a recommended injection order to minimize impact of carryover
    - standards low to high, samples in sequence of collection. For preclinical work, inject in ascending dose order in order of collection
- Contamination (peaks in blank) should trigger an automatic investigation.
  - Acceptance of run results should be dependent on results of investigation

Matrix Effects

- Assessment required for MS assays
- Assessment should be based on internal standard normalized results
  - absolute effect is not relevant if variability is compensated for by internal standard
- Causes and reduction should be studied during method development and processes to reduce/minimize should be developed
- Number of lots for assessment
  - pre-clinical
    - male/female/hemolyzed (female pre-clinical matrix may be dependent upon time during menstrual cycle plasma was collected)
    - strain/age agnostic
  - clinical
    - 2M, 2F, 1 Hemo, 1 lipemic (use scientific judgment to determine if additional is needed)
- Procedure for assessment and acceptance criteria
  - variability of analyte/internal std ratio across lots
    - 15% CV acceptance
  - perform assessment at low and high concentrations

Matrix Effects (cont.)

- Impact of dosing vehicles
  - monitor during sample analysis and use best scientific judgment
- Impact of analog vs. stable label istds
  - stable label assays generally less prone to effects
  - use of analog may dictate need to assess absolute matrix effect of analyte and istd to determine if similar or different
- Metabolite and/or co-med impact
  - issues should manifest during selectivity exps. No need for multiple lots
- Regularly assess effects during routine analysis
  - through istd variability
- No need for specific phospholipid assessment during validation
  - should be assessed during method development
- Calculations
  - relative ME= variability of the ratio of analyte to internal std across the different lots
  - absolute ME= ratio of analyte response in post extract controls vs. neat