Outcomes of the Global Bioanalysis Consortium's Recommendations: Large Molecule Discussion Topics

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Global Bioanalysis Consortium
On harmonization of bioanalytical guidance

TEAM LEADS

TEAM		Team Lead
L1	Specific run Acceptance	Marian Kelley
L2	Assay Operation	Lauren Stevenson
L3	Assay Formats	Sherri Dudal
L4	Reagents, stability	Lindsay King
L5	Automation	Scott Davis
L6	Immunogenicity (effect on PK)	Jeff Sailstad

L1 - LBA Run Acceptance

Consensus achieved on:

- Use of Method Total Error during pre-study validation to set in-study QC acceptance criteria
- Prepare, qualify and freeze aliquots of standard curve calibrators. Establish stability by comparing fresh vs. frozen standard curve calibrators during A&P.
- Quality Controls should be prepared separately from calibrators, qualified and frozen to mimic the study samples. QCs should always be tested from the frozen state.

L2: Selectivity (Lipemic Samples)

Recommendation

- The need to perform selectivity assessments with lipemic samples will be dependent upon the drug, disease indication and assay format
  - Typically, performing selectivity assessments with disease matrix samples will address any effects of lipemia which may be present in that population
  - The team is actively seeking examples where there was an issue caused by lipemia to guide when additional assessments may be recommended

L2: Selectivity (Hemolyzed Samples)

Recommendation

- The need to perform selectivity assessments with hemolyzed samples will be dependent upon the characteristics of drug, its target, disease indication and assay format
- The team is actively seeking examples where there was an issue caused by hemolysis to guide when these assessments may be recommended
  - Examples gathered to date indicate that issues due to hemolysis are rare and have not been observed with mAb therapeutics. However, insulin and related therapeutics are more likely to be sensitive to hemolysis

L2: Parallelism

Routine parallelism assessments currently not being broadly implemented industry-wide (some/few are doing routinely)

- Currently, more questions than answers around when to perform the assessment, how to perform it, and how to report the data

Recommendation:

- the need to perform parallelism assessments will depend upon the characteristics of the drug, its binding partners and specific assay reagents

The team continues to seek examples where non parallelism has been observed to guide when assessment may be recommended

- Examples gathered to date indicate that non-mAb therapeutics, especially peptides, may be more likely to have issues of non parallelism
- No examples yet identified for mAb therapeutics

L3: Non-plate based Assay Platforms

Sample testing is run in series on some platforms rather than plates

Recommendation:

- Singlet analysis of samples can be run as long as the CV is within an acceptable range developed during assay validation.
- A run is not limited to ’96’ in this case.
- short-term stability data will be used to determine the time required for ‘new runs’ to be started and drift effects if applicable
- intermittent QC sets during the sample analysis
- A standard curve can be added in at the start of every run.

L3: Cell based assays for PK

More extensive method characterization is required due to the sensitivity of the assay to numerous factors:

Recommendation for assay characterization:

- serum lots, cell passages, the length of time in culture conditions
- the reproducibility during pre-validation will determine if the samples can be run in duplicate or triplicate.
- sample analysis requires that QCs be placed within the plate as well as along the edge so that any border effects can be determined.
- LLOQ, LQC, MQC, HQC, and ULOQ precision and accuracy criteria up to 30% and total error up to 40% may be required.
- The ISR guidelines would also be wider with a 40% margin.

L3: Cell based Assays for PK

Recommendations for cell line stability:

- Cell passage and freezing stability need to be assessed. Within the same run, QC performance (%RE ≤30%) assessed against calibration standards in both “fresh” and ‘recently frozen’ cells.
- Ranges for parameters such as assay signal, cell growth rate, and viability should also be considered as appropriate.
- Batched working banks performed under single campaign may require qualification of each batch.
- New working banks established outside of validation require qualification.
- Critical Reagents to be assessed: assay performance with multiple FBS/FCS lots/sources and different media should be evaluated.

L4 - Changing Critical Reagents

Consistent questions around

- number of runs
- number of QCs for lot changes
- changing critical reagents and stability assessments.
- Some groups like multiple runs and high QC number other prefer one run and two QCs

Following are a) some of the key recommendations and

b) some of the most controversial recommendations

L4: Reagents and their stability

Key Draft Recommendations

Recommendations:

- Create an SOP or similar document that defines reagent requirements including how, what, where and when. Documentation is required to ensure a consistent and reproducible process.

- define how to test reagent stability, who will do what test, where the data will be stored, what criteria will be used, where to document this work.

- sufficient characterization to enable consistency/process control. Including: Identity, source, purity, concentration (or titer), binding affinity, isotype (Mab/polyclonal), MW, specificity, incorporation ratio, aggregation level, storage

Feedback requested and received to this. Degree of characterization must case by case.

L4: Reagents and their stability

Selected draft recommendation with concerns

Changing Critical Reagents

Recommend test performance in assay as follows:

- Single Critical Reagent Change – min 1 reagent qualification run in parallel with current/original lot. Include 5 QC levels and max response as an acceptance criteria.
- Single Critical Reagent Change and no overlap with current /original lot – min 3 independent reagent qualification runs. Include 5 QC levels and max response as an acceptance criteria.

Consistent questions around number of runs and number of QCs for lot changes, changing critical reagents and stability assessments. Some groups like multiple runs and high QC number other prefer one run and two QCs

L4: Reagents and their stability

Selected draft recommendation with concerns

Acceptance criteria based on QCs does not address the potential for significant difference in maximum response despite acceptable back calculated QC values.

Recommendation:

- acceptance criteria for both new lots of reagents and for stability testing include maximum response.

Feedback varies for use of max signal/ signal from no to yes or only appropriate in specific circumstances (eg ADA).

Many questions re practicality/applicability for some platforms despite caveats in recommendations above.

Monitoring QC signal may be more easily adopted as a tool rather than **as acceptance criteria.**

L4: Reagent Stability

Recommendation:

- Assign test/retest rather than expiry dates to define critical reagent stability initially.

  Generally very well received but one group strongly disagrees

Recommendation:

- Define how to test reagent stability, who will do what test, where the data will be stored, what criteria will be used, where to document this work.

  Generally very well received some disagreement due to concern that would require a lot of extra work.

L5: System Documentation

- Standard Operating Procedure (SOP)
- Installation Qualification (IQ)
- Operational Qualification (OQ)
- Performance Qualification (PQ) (If Significant Software Component)
  - User Requirements
  - Validation Plan
  - Test Worksheets
  - Validation Summary
  - Traceability Matrix
  - Test Incident Log
- Change Control (If Applicable)
- Configuration Only Change Control (If Applicable)
- Configuration Management

L5: Validation of a Modular System

- Must be done **before** assay validation
- Should be included in original PQ for automated system
- No need to re-validate anything already validated
- Includes integration of LIMS in process
- If module added after PQ, there are three options:
  - Generate IQ/OQ/PQ documentation as defined above for automated systems
  - Generate a Change Control to another validation
  - Perform as part of validation for another system (including analysis systems)

L5: Accuracy and Precision Testing

- Scenario 1: Manually validated assay (*benchmark only, out of scope of committee*)
- Scenario 2: Assay validated with robotics
- Scenario 3: Manually validated assay along with assay validation with robotics
- Scenario 4: Manually validated assay followed by assay validation with robotics LATER

L5 : Automation Practices in Large Molecule Bioanalysis

System Documentation

- SOPs
- IQ/OQ/PQ
- Change Control
- Configuration Management

Validation of a Modular System

Accuracy and Precision Testing

- Multiple scenarios exist depending on the assays

L6 - Immunogenicity (effect on PK)

Understanding PK and immunogenicity - a regulatory expectation concerning interference

Impact of ADA on PK evaluation

- Real effect (effect of ADA on clearance of drug)
- Artificial effect (effect of ADA on PK assay)

Why are the PK methods sensitive to ADA presence

- Compounding (?) factors: format, modality, type of response; isotype, affinity, avidity, titer level, dynamic response maturation, transient vs. persistent, pre-existing Abs
- Bioanalytical strategies to address ADA impact on PK
- Investigations - Risk based approach in conducting investigations