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

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# TEAM LEADS

| TEAM | | Team Lead |
|---|---|---|
| L1 | Specific run Acceptance | Marian Kelley |
| L2 | Assay Operation | Lauren Stevenson |
| L3 | Assay Formats | Sherri Dudal |
| L4 | Reagents, stab tiered | 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: Large Molecule Specific Assay Operation

- Not recommending routine analysis of lipemic/hemolysed samples in validation
- Not recommending routine parallelism assessments

# L3 - Assay Formats

- For platforms where samples are run in series rather than on plates,
  - singlet analysis of samples
  - A standard curve can be added in at the start of every run.
- Cell-based assays for PK assays

# 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

# L4 – Acceptance Criteria based on QC responses

- Significant range of feedback on use if max signal/ signal from no to yes, to appropriate in specific circumstances (e.g. ADA). Many questions re practicality/applicability for some platforms despite caveats in recommendations above.
- Monitoring QC signal, since we already generate this data, may be more easily adopted as a tool rather than as acceptance criteria.

# L4: Reagent Stability

- Assign test/retest rather that expiry dates to define critical reagent stability initially.
  - Generally very well received but one group strongly disagreement
- Define how to test regent 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 : 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

# Back Up Slides

# 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

# L2: Parallelism

- 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

- For platforms where samples are run in series rather than on plates, singlet analysis of samples can be run as long as the CV is within an acceptable range developed during assay validation.
- The entire run is not limited to ’96’ in this case as plates are not used for reading.
- Short-term stability data will be used to determine the time required for new runs to be started as well as any drift effect which will be measured by 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

- For these assays, the method validation report requires more detail due to the sensitivity of the assay to numerous factors such as serum lots, cell passages, the length of time in culture conditions, and so on. Additional cell line performance parameters such as signal, confluency, and viability should be considered as well.
- The reproducibility during pre-validation will determine if the samples can be run in duplicate or triplicate.
- Also, 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

- 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 “stability” 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: assay performance with multiple FBS/FCS lots/sources should be evaluated. Media and supplement sources may also impact assay performance.

## Draft Recommendations For Comment

Overall feedback to date on the recommendations has ranged considerably reflecting perhaps the range of practices and users needs.

Some have felt the recommendations are too specific but more often we have had a request for more specificity, details, clarification and/or examples.

Some confusion/questions regarding reagent testing/characterization using the assay(s) of intended use for sample analysis versus testing independent of assay

Following are
a) some of the key recommendations and
b) some of the most controversial recommendations

# L4: Reagents and their stability

## Key Draft Recommendations

**Recommend an SOP or similar document that defines reagent requirements including how, what, where and when. Recognize that documentation may take many forms. Documentation is required to ensure a consistent and reproducible process.**

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

**Recommend sufficient characterization to enable consistency/process control. Recommend) - 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.**

## 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.
- Eg second biotinylation of a monoclonal from same lot of purified Ab as use initially
- Multiple Critical Reagent Changed – min 3 independent runs. May require some degree of re-validation for Regulated work. Include 5 QC levels and max signal as acceptance criteria.

**Consistant 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 solely on QCs does not address the potential for significant difference in maximum response despite acceptable back calculated QC values. *Thus we have included the recommendation that acceptance criteria for both new lots of reagents and for stability testing include maximum response*.**

**Significant range of feedback on use if max signal/ signal from no to yes, to appropriate in specific circumstances (eg ADA). Many questions re practicality/applicability for some platforms despite caveats in recommendations above.**

**Monitoring QC signal, since we already generate this data, may be more easily adopted as a tool rather than as acceptance criteria.**

# L4: Reagents and their stability

## Selected draft recommendation with concerns

**Reagent Stability within the assigned stability period. Recommend assign test/retest rather that expiry dates to define critical reagent stability initially.**

**Generally very well received but one group strongly disagreement**

**Recommend define how to test regent 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. This is a general concern when new process are proposed.**

# 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

# L6 team

1. Introduction-
    - What is GBC, what we are trying to accomplish.
    - Address paper customers – BA and PK scientists, what can they learn from the paper
2. Understanding PK and immunogenicity - a regulatory expectation concerning interference
3. Impact of ADA on PK evaluation
    - Real effect (effect of ADA on clearance of drug)
    - Artificial effect (effect of ADA on PK assay)
4. The analytical methods
    - Brief description of PK assays for biologics
    - Brief description of ADA methods, Reference white papers to cover assay developments and validation
5. 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

# The Outline - continued

- Bioanalytical strategies to address ADA impact on PK
  - Investigational Approaches to determine real effect or bioanalytical artifact

    As part of method development:

    Post method development, unexpected results, what to do to understand what does it mean?
  - Assay modifications to address bioanalytical artifac
- Other than BA strategies to address ADA impact
  - PD indicators
  - Efficacy indicators
- Investigations - Risk based approach in conducting investigations –
  - Case Study - preclinical –
  - Case Study - clinical –
  - When do you investigate when do you conclude it doesn't matter?
  - How much proof do you need to defend your decisions?
- Conclusion and Recommendations