Roundtable Session 2 – Table 3 - Learnings from Bioassay Transfers

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Abstract
Bioassays can exhibit differences in performance between analysts and/or between labs. This can complicate method transfers, trigger investigations, and require method updates. In some cases, the root cause turns out to be a seemingly minor method or handling detail with an unexpectedly large impact.

Discussion Questions:
1. What have you observed regarding analyst-to-analyst or lab-to-lab variability in bioassay performance?
2. What have you learned from the bioassay transfers you have been involved in?
3. Have you made any surprising observations or noticed any recurring themes?
4. How can we improve bioassay robustness so that method transfers run more smoothly?

Additional questions:
- From the HA perspective, what are the most common "dos and don'ts”?
- Have you noticed any impact on cells or the assay, when using different equipment at the sending unit (SU) and receiving unit (RU)?
- Are the method transfers usually internally or externally, at which phase do you usually transfer
- What would you do if your requirements do not match RU do you adjust the SST criteria to accommodate the difference?

Notes:

1. <u>Communication & Knowledge Management</u>
- Mostly minimal knowledge transfer – comprehensive knowledge transfer is very time and resource consuming:
    - SME Engagement: Direct analyst-to-analyst and/or SME-to-SME communication is vital for capturing details often omitted from formal SOPs.
    - CRO may remove specific technical details from SOPs to maintain operational flexibility. Sending Units (SU) should insist on retaining critical technical details, e.g. specific pipette types, exact mixing counts ("mix 13 times"), critical consumables/reagents (plates etc.)
- Visual Documentation:
    - Utilizing cameras (e.g., GoPro) to record assay steps facilitates training and helps identify subtle analyst behaviors that impact results. Preservation of knowledge by recording analyst A, then the training can be passed directly to other analysts from analyst A
- On-site training of RU (receiving unit) analysts by SU (sending unit) analysts if possible.
- Data Transparency: Full access to raw data from RU is important for understanding and solving issues.

2. <u>Technical Variables & Gap Assessment</u>

- Equipment Disparities:
    - Significant variability in plate reader generations or different device manufacturers can impact sensitivity, detection limits, signal-to-noise or maximum signal -> feasibility tests recommended
    - Bias can also be observed between same device models, plate readers are not identical, minor details in setup or alignment may already impact results
    - Incubator environments (differences in CO2 and humidity limits) may directly impact cell growth and signal-to-noise ratios.
    - Good gap assessment is key, if additional equipment needs to be acquired for RU, plan for additional time
- Consumable/reagent Bridging: Changes in manufacturers or lot-to-lot variability in consumables/reagents may require formal bridging studies to maintain assay stability. Examples:
    - Slight changes in coating of the plates impacted assay results
    - Changes in embossing letters and numbers for rows and columns impacted the plate layout and thereby shifted the dose response curves
- System Suitability Tests (SST): Adjusting SST limits to accommodate site differences may be necessary but understanding why there are differences in SST limits is important, there might be underlying issues. Questions to consider: Do the "new" SST limits still holistically control the product quality? It also may not always be possible (e.g. commercial products) and would require significant regulatory justification.
- If possible, test Robustness at the Receiving Unit: method may not behave identically in a new environment.

3. <u>Transfer Strategy & Execution</u>
    - Proactive vs. Reactive: Transfers are frequently reactive (troubleshooting after a failure). Investing time in a thorough initial training phase may prevent costly delays later.
    - Bridging & Statistics: Late-stage transfers for regulatory filings require large, high-quality datasets. Statistical equivalence testing of various batches seems to be a common approach over multiple testing of single samples. The sample size may depend on the availability of batches
    - External and internal transfers (e.g. development lab to QC lab) seem to occur equally, sometimes even mixed approaches dependent on project stage, however internal transfers are considered simpler and usually less time-consuming due to established processes.
    - Timing of transfers, early-stage vs late stage: no clear preferences

4. <u>Regulatory, Quality, and Logistics</u>
    - Assay Lifecycle: Bioassay transfers should be viewed through the lens of the analytical lifecycle (ICH Q14), regular assessment of suitability of assay
    - Cross-functional Alignment: Pre-alignment with QA and Regulatory teams are important to approve the transfer strategy and maintain good quality oversight.
    - Timeline Pressures: Analytical timelines are often the first to be compressed when manufacturing process transfers face delays, placing undue stress on the SU/RU laboratory.
    - Contractual Clarity: Good quality contracts and clear communication of deliverables are necessary to maintain good quality oversight and achievement of timelines
    - Early Regulatory Consultation for complex issues to define clear strategy may be an option, however consultations on method transfers are not the norm
    - Consider change control process for any changes

Lessons Learned

- Good communication is key
- Standard Operating Procedures rarely capture the nuanced "tricks" of the trade. Use video documentation and SME exchanges to bridge this knowledge gap.
- Rigorous Gap Assessment: Conduct a formal comparison of equipment specifications (plate readers, incubators, reagents etc.) to identify potential environmental biases.
- Quality Agreements are Essential: Ensure internal and external agreements, explicitly define the scope