Roundtable Session 2 – Table 5 – How Are You Monitoring the Health of Your Bioassay Methods?

How Are You Monitoring the Health of Your Bioassay Methods?

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Abstract:

Monitoring of bioassay performance is an important part of analytical method life cycle management. Routine monitoring provides an understanding of performance, maintenance of appropriate method control, proactive identification of potential method issues, and can provide data that may support product quality or process capability evaluation.

Discussion Questions:

1. What constitutes bioassay method "health"? What parameters are important to monitor to assess bioassay method performance?

2. How does the approach change based on the phase of development?

3. What are potential use cases of bioassay method monitoring that may be utilized to evaluate product quality? Or process capability?

4. What are some of the challenges for bioassay method monitoring?

5. How are AI and other digital tools influencing practices?

Notes:

Question 1. What parameters are important to monitor to assess bioassay method performance?

- As you execute the method how do you ensure the method is performing well?

- EC50, slope, reference standard performance, assay control, also take into account relative potency of control to reference, Trend SSDs based on lot number and site and other critical reagents over time.

- Definitely want balance between essential and trending/dependend reagents.

- Accuracy and Precision identified through qualification but development work identifies the importance of things like lot number for CTG or FRTU bank etc.

Method performance monitoring program – What does that look like across our companies? What is different and what is the same?

- If you work with a CRO it becomes a business decision about what parameters and details you want them to track and provide information for which can be a problem from a finanical standpoint.

- You may not know what you need to trend until a problem comes up and an investigation comes in.

- Some companies just start with the more is better, providing a locked template to the CRO.

- What are some of the parameters from early development that you inform your CRO about?

- Use of platform parameters

- Use CRO and they are required to document and track all info, send data back upon request

- Mentioned that they even some request lot numbers of flasks and media

- For bioassay groups that don't use EC50 as part of System Suitability and have their own QC groups with no CROs they track A,B,C,D parameters.

- Critical reagents stated in the method are all qualified before use and monitored but is rigorously tested prior to use. This group doesn't use a control but has intraplate replicates.

Questions about if you don't use a assay control material how can you track if your reference material is trending in one way or another? Restricted to one plate as a small company so unable to do that.

Questions about when to implement the control material separate from reference material? Can you generate the material and have both produced before FIH? Depends on the ability and capacity of process scientists.

Reference standard has to be comparable to your product. So if major process changes are made later does the reference needs to be changed?

The responsibility of reference material is to be associated with the performance of the method, it does not change if your product undergoes process changes?

Assay control samples, how wide can the acceptance criteria be for the assay?

Most use 70-130%

What limits should be set for assay control?

Depends on the capability of your method? Assay specific.

Trending of materials,

Methods running at 6 different CRO? Considered equivalent. But everytime you needed new primer pairs you would needed to see a shift and that historical knowledge strengthened their confidence that certain primer pairs were off or if this is an acceptable amount of change from one primer pair or another.

Since this changed so often then you don't want to qualify the primer pairs.

New primer probes need to be qualified at another company but the acceptable variation is trended and accepted.

Early development - reagent trends,

Are there other approaches that you are seeing that need to be modified as you move through development to late stage?

What is the purpose of assay control was discussed to set a baseline for the group. The earlier you can put a reference standard be put in place the better. Requires some level of compariability (cannot use research grade material, typically use TS1). Once several batches have been made, requesting excess production or retains that could be an option. Building requests for reference into the sampling plan is a great strategy. Discuss the option to pool batches with less inventory but then recharacterizing the pooled product. Request for 3-5 year supply. Discusion about diluting from manufacturing conc to useful conc in order to create more reference material.

Challenges around storage of reference material. Option for off site storage.

Have you encountered use cases where your performance trending has been useful for product quality discussions or issues that have arose over time in variability?

Stability questions inevitably arise and this control charting can help answer if dips or trends in stability are real or method issues.

Have seen bias in trends that help investigations. Position effects, changes in plate layout to account for those issues and then had to revalidate the method. DP batches were seeing vial to vial variability in potency. Did it impact precision and accuracy.

What is due to method variability and due to process variability? How can you tell the difference?

Does the reference lot need to be representative of assay control.?

Input from a table member from a company using gene therapy - small batch production size,

Request to pool several batches together to be used as the reference control to account for variability.

Produce individual batches, no reference but have representative model that is process comparable and can use that material for testing of each batch. Accepted by authorities so far. Requires wider ranges of acceptable criteria for the method. Did you add control strategy components to account for the lack of reference?

^What parameters are you monitoring if you don't have a batch specific reference in your potency method? Trending of the reference material to measure assay performance. Plasmid DNA product.

How are digital tools being used to help you monitor your method performance?

- No data review of trending so adding a script that pulls data out of LIMS into JMP and then applies historical limits.
- Used ChatGPT to design code for script.
- Automated export from SMP to LIMS to JMP
- Alignment of code and nomenclature for the script step 1, a single script for all sites to use, and then data is aggregated into Tableau and anyone can trend and graph the data from all sites, took ~ 4 years but the hardest part was getting all users to align on the nomenclature around the script
- Issues around if you have multiple samples sets into one ELN
- Some people use PLA software for control charting
- MultiSite trending allows you to highlight problems as they arising in real time and allows you to get ahead of it before it's a full investigation

Are you reviewing method performance trending on a consistent basis?

- Data from CRO every 6 months, upon request
- Challenges for the CROs to comply with request for data every quarter
- Trending is a hard sale, like flossing, its boring 95% of the time so people just don't want to do it since its not satisfying
- CROs do trending but they don't share the data, its communication gap
- Inconsistency with file naming and nomenclature across analysts make aggregation and trending more complicated
- Data cleaning system initiated so that the format for naming is consistent across all users
- Data structure is important and hard to maintain consistency
- Some members where monitoring yearly

Do you include your qualification data in your control charting?

- Yes
- Increases testing and data points since qualification is early phase
- May be helpful to highlight them as a stand alone but most people do include it
- Guideline say if you have lots of data you don't need it as much as if you are early on.

When trending is slightly going up or down +/- 10% why are you not worried?

- Because you should see an upward trend followed by a downward trend. Give it time, focus it early, possibly retest if you see the treding continue. but if it doesn't correct then you should worry.

When should you begin to worry and how do you set your out of trending limits?

- If we have Ras do the analysis for us, they can make notes and denote outlier or singe event one offs or if you they recommend escalation.

Assay control issues and edge effect, but want to maximize the plate use? Can you put the Reference and control on the edges of the plate. (They have a moat and only need 6 data points)

- As long as there is consistency and you keep it consistent and you have
- Snake Method
- Randomization by TECAN
- Inverted dose series on half the pate
- Moats or no moats

Question about the reference standard - Early phase set up for N. How do you determine how many n's you need?

- Why not use a Qualification data for the trending?
- Number of reportable values is based on precision calculated in the Qualification report.