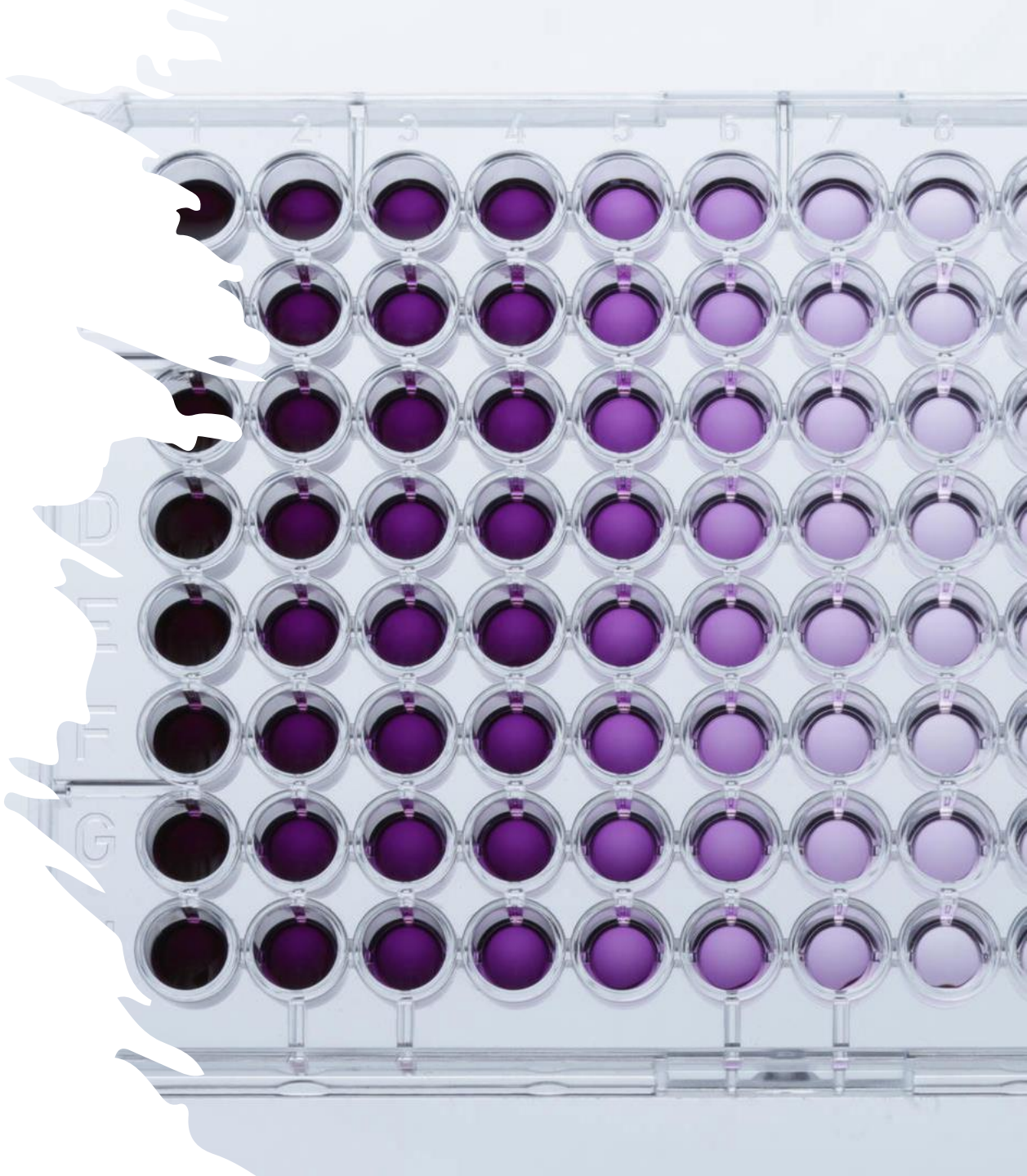
The *Special* World of Bioassay

Approach/Setup, Special Situations and Keys to Success

Ned Mozier Keynote for CASSS Bioassay Conference 2025



Abstract

Bioassays in the biologics industry largely represent an evaluation of a product's ability to exert an in vitro biological response that represents the intended mechanism of action in humans. The goal is to confirm that in each test sample that the molecule has maintained the critical structural features necessary for its actio
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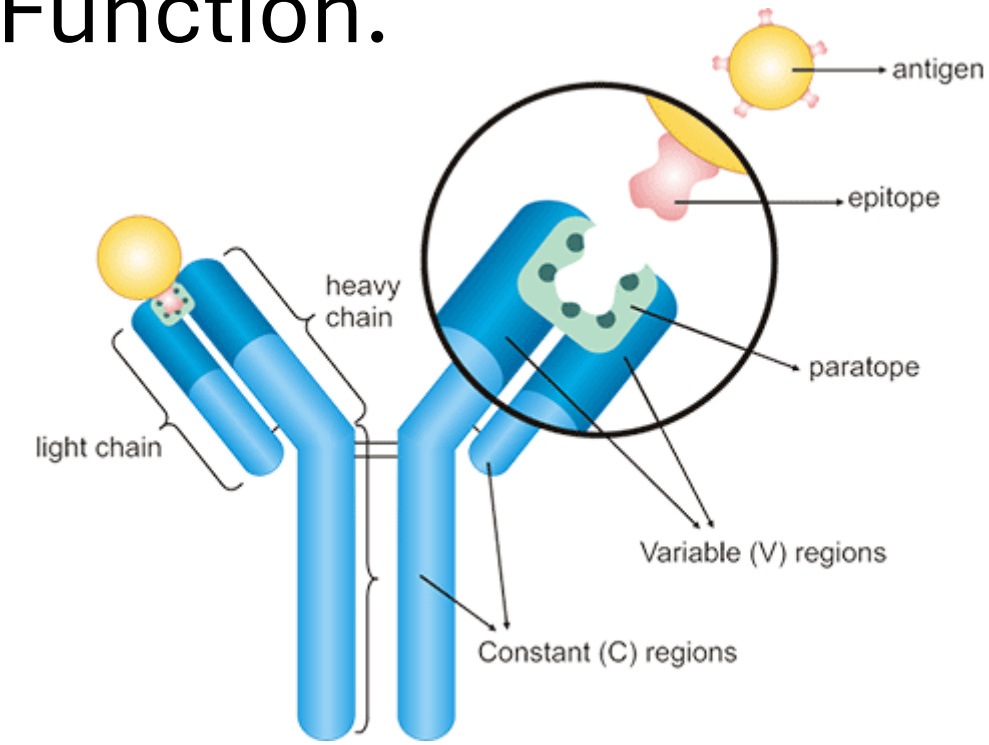
specialized field to innovate, adopt best practices, and deliver crucial information about our potentially life-changing drugs in development. It is incumbent on us, as practitioners and experts, to not only produce high quality data, but to develop a language to clearly and simply explain the significance of bioassay results.

Bioassays are typically the only test on a product specification list able to detect *meaningful conformational* changes that may arise based on the manner produced or the conditions of storage.

Why is Bioassay “special”? It is the only Product Evaluation of 3D structure vs. Function.



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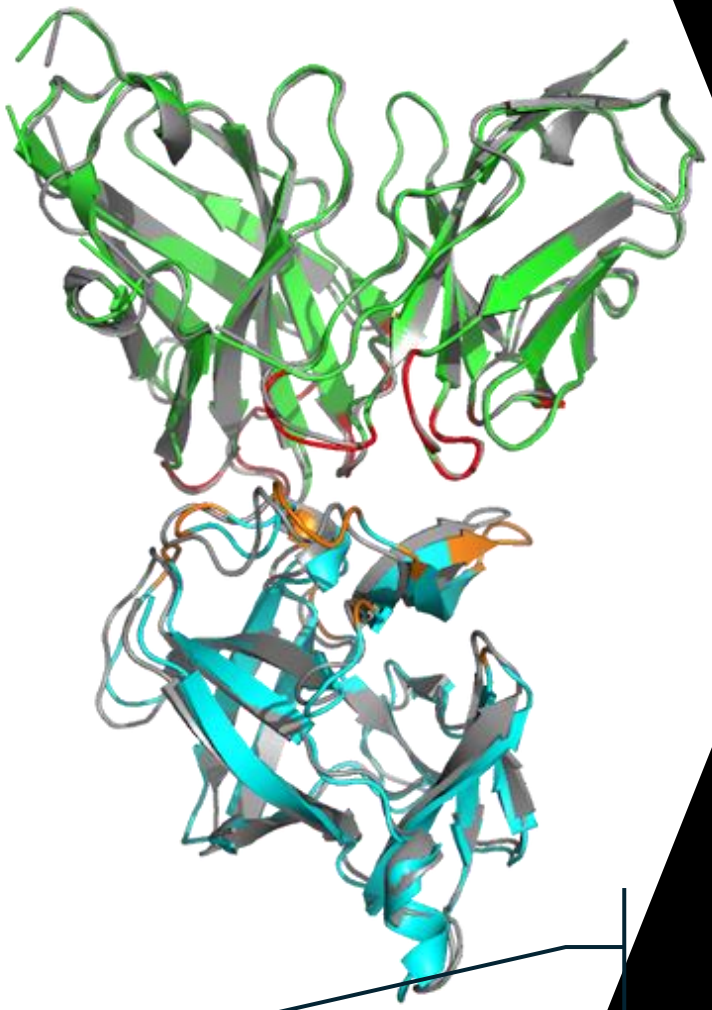
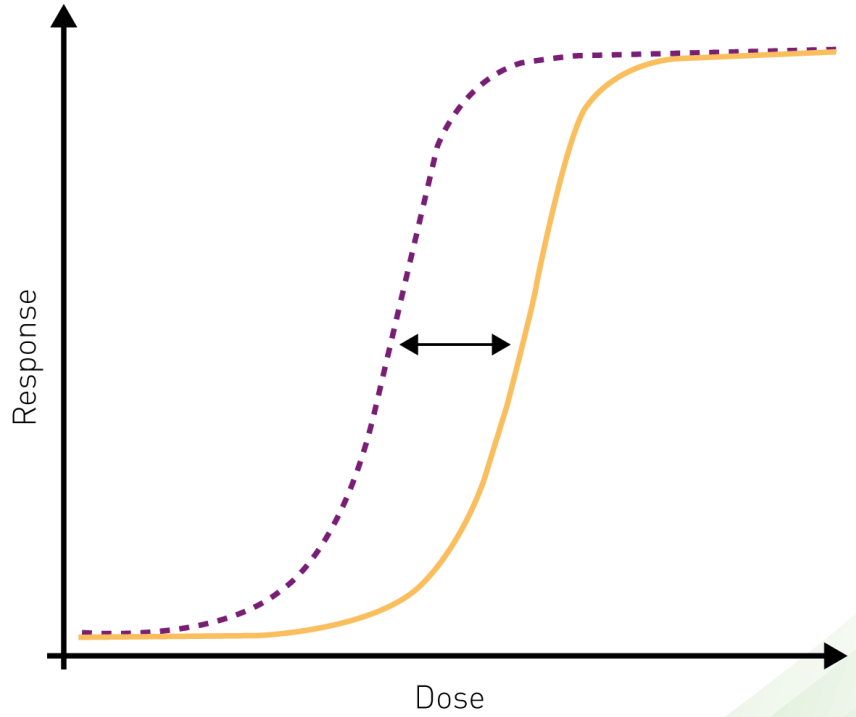


Sometimes considered a “catch all” assay for those rare instances where the product has changed in a way that is “missed” by the more conventional physico-chemical test methods

Is the effort worth all the testing we do?

Topics

- Establishing the basics & understanding terminology
- Getting Started: Developing a Test Method
- Bioassay lab essentials
- Managing “noise” / impact to specification limits
- Areas of Focus
- Using Stability Studies Wisely
- Successful bioassay strategies
- Correlation of Assays
- Tips / lessons learned
- Key Points



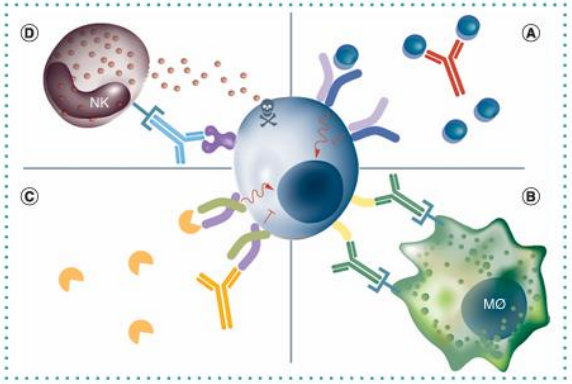
Out of scope for today: Animal assays, vaccine potency assays, cell types, automation, test method validation

First let us establish some basic principles

- What do we call it?
  - Bioassay = **Relative Potency Assay (RPA)**
    - Always *in vitro*, never animal, typically cellular, sometimes binding only
  - Potency Assay – confusing, this term can mean many things
- What is ultimately needed for CMC (chemistry & manufacturing control)?
  - Confirming each batch is the same or similar enough to those used to establish clinical efficacy
    - Collaboration with discovery unit / molecular designer
  - Confirming that preferred stability condition prevents meaningful change in product's ability to deliver the intended mechanism of action (MOA)

Getting Started: Developing a Test Method

- Select a well-characterized reference standard
  - Typically the drug substance (DS) used to make the lot of drug product (DP) used for Phase 1 clinical studies
- Choose an assay or assays which model the intended MOA(s)
  - Non-cellular (i.e. immunoassay) is viable for early development
    - Avoids over-investment for products, few of which will actually reach Phase 3
  - A cell-based RPA is typically required by health authorities to support Phase 3 / pivotal clinical trials
    - Cell types / performance needs to be locked in
  - Each MOA to be claimed for the product needs its own RPA
    - E.g. for a monoclonal antibody (mAb) with effector function, the Fab target binding and the Fc functionality need to be monitored (multiples may be required for for biosimilars)
- Significant Development required – the earlier the better
  - For a Quality Control environment, significant investment needed
  - Assay performance characteristics need to be well understood (i.e. a great deal of testing)
    - Establishing specification limits becomes increasingly important as the product advances in development



White et al., 2019, biotechniques

By Phase 3, you will be nearly fully invested!

Investments Needed for the bioassay lab

- Facilities / specialized equipment
  - Incubators, plate readers, freezers (including liquid N2), *ample* hood and bench space, specialized software, liquid handling devices, pipets galore
- Personnel / skills
  - Method / validation scientists
  - Skilled technicians
  - Experienced cell biologist
  - Data / software curators
  - Statistician(s) familiar with bioassays
  - Robotics / liquid handler expertise
- cGMP infrastructure (if supporting Phase 3 and beyond)
  - Though more expensive, conducting tests cGMP **earlier** in development pays dividends when transferring to commercial lab in later stages

Pipetting and reagent/cell handling are one of the most underappreciated natural talents in our industry – some have it, some don't, some can learn, some cannot. Know how to know the difference and deploy talent appropriately.

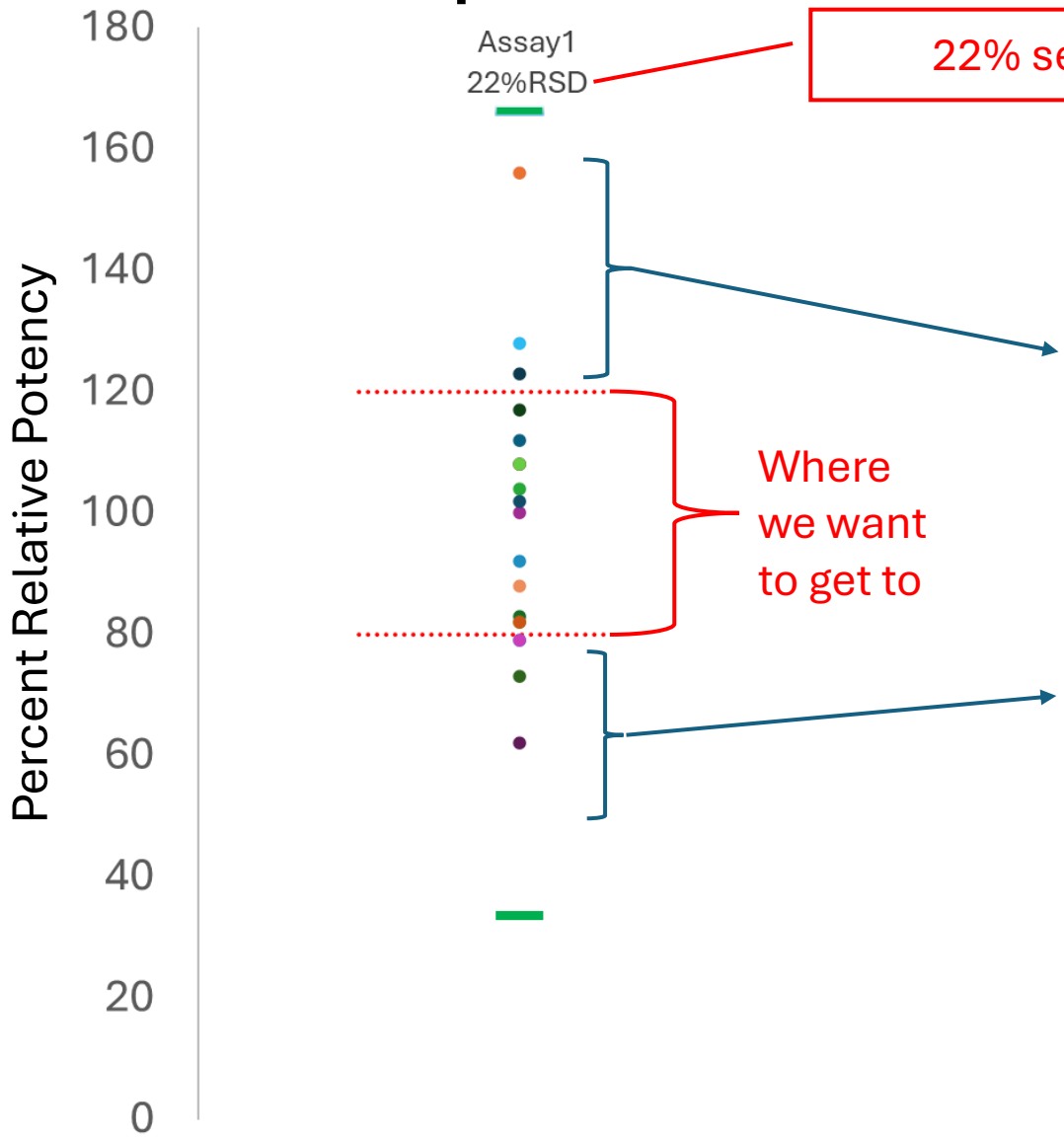
Once the assay reagents and cells are confirmed to measure the intended MOA, Controlling assay “noise” is necessary to achieve a target specification

- Define the range and probe the dose response curve
  - Obtain lower and upper assymptopes
- For early development, target specification range
  - 50-150% may be acceptable
  - 70-130% is generally acceptable
- For later development (Phase 3 and beyond)
  - 75-125% may be acceptable
  - 80-120% (or 80-125%) generally acceptable

Look for correlations to other attributes (post-translational changes, degradation processes observed from other test methods during stability studies

Once the Method is Established, Testing the Product against Itself is a simple and effective development tool

test1	108
test2	156
test3	83
test4	79
test5	100
test6	82
test7	123
test8	108
test9	117
test10	112
test11	62
test12	73
test13	92
test14	88
test15	104
test16	128
test17	79
test18	108
test19	102
test20	82

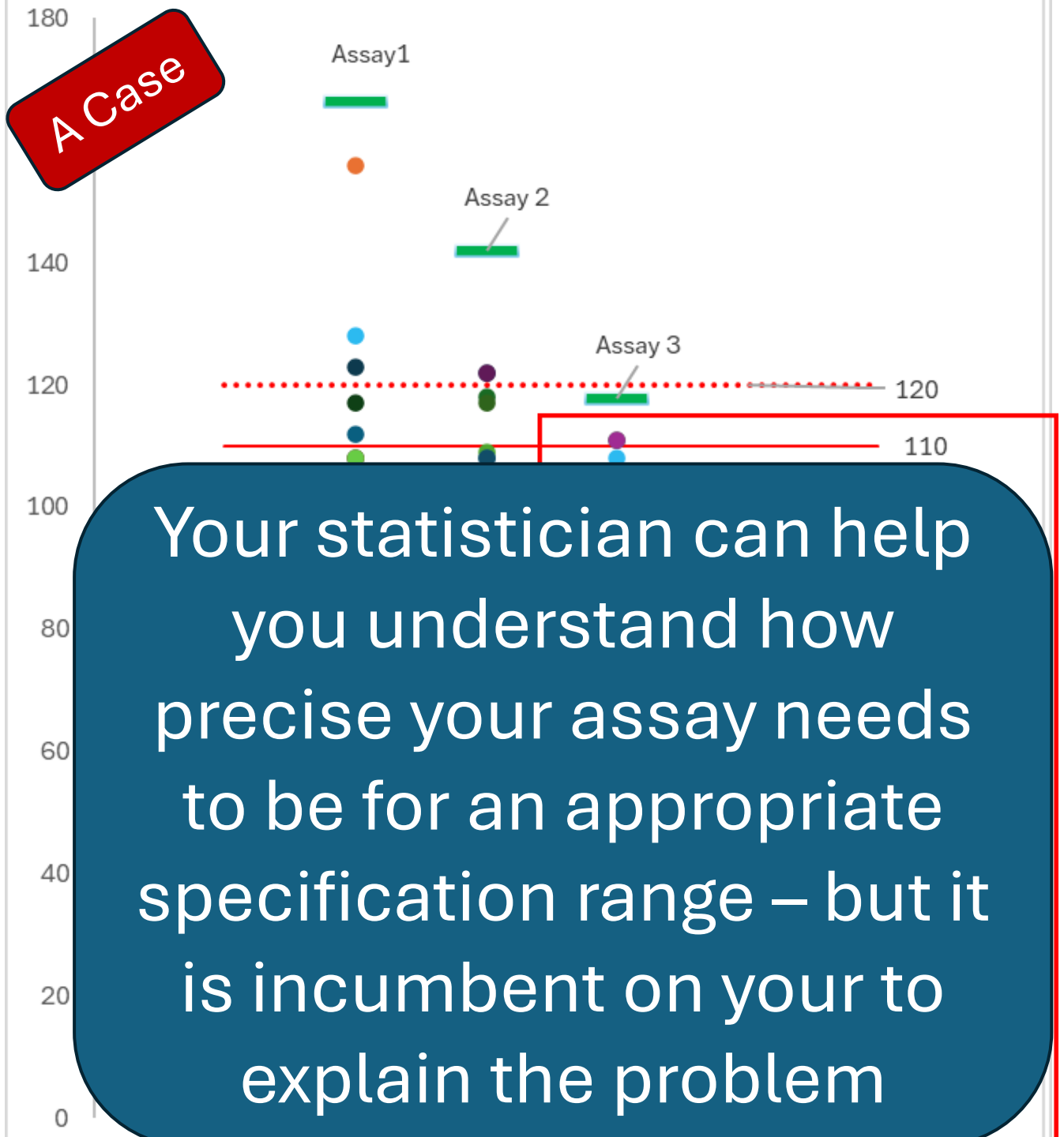


Understand what is causing these results that are very high or very low

1. Analyst?
2. Cell incubation conditions?
3. Reagent?
4. Edge effect?
5. Cell handling?
6. Incubator / hood?
7. ???

How Much Variability is too much?

- Goal: Getting 99% confidence interval within target limits
- Know how low you will need to go
- Minimal Qualification needed to support a Phase 1 study
  - Reasonable effort to reduce noise needed – specifications may be dictated by capabilities
- Phase 3 and beyond: It is expected that assays will improve, and specifications will be narrowed if possible (data driven)
  - Know expectations of health agencies



Balancing the Release Test (%RP) Limits with Assay Capabilities: A Strategy

- The Goal is largely binary: Pass "good" batches, Fail "bad" ones
- Define "good" enough – result is "within assay noise"
  - Caution: Individual variation in numerical values within this range to be ignored (more later on this)
- Discussions on final specification limits come after optimization of the bioassay (reducing noise as much as possible)
  - At some point there is no further to go, there are unknown unknowns currently in the world of biology – this is "inherent" variability
- Develop training tools – understand why some analysts are better than others, become a learning organization
  - Create training modules to evaluate analyst capabilities
- Once optimized, qualify or validate: Design a protocol that captures all necessary parameters
  - Establish the key parameters for reference standard performance and monitor those same ones going forward when testing product
  - This will help greatly in the future when investigations are needed

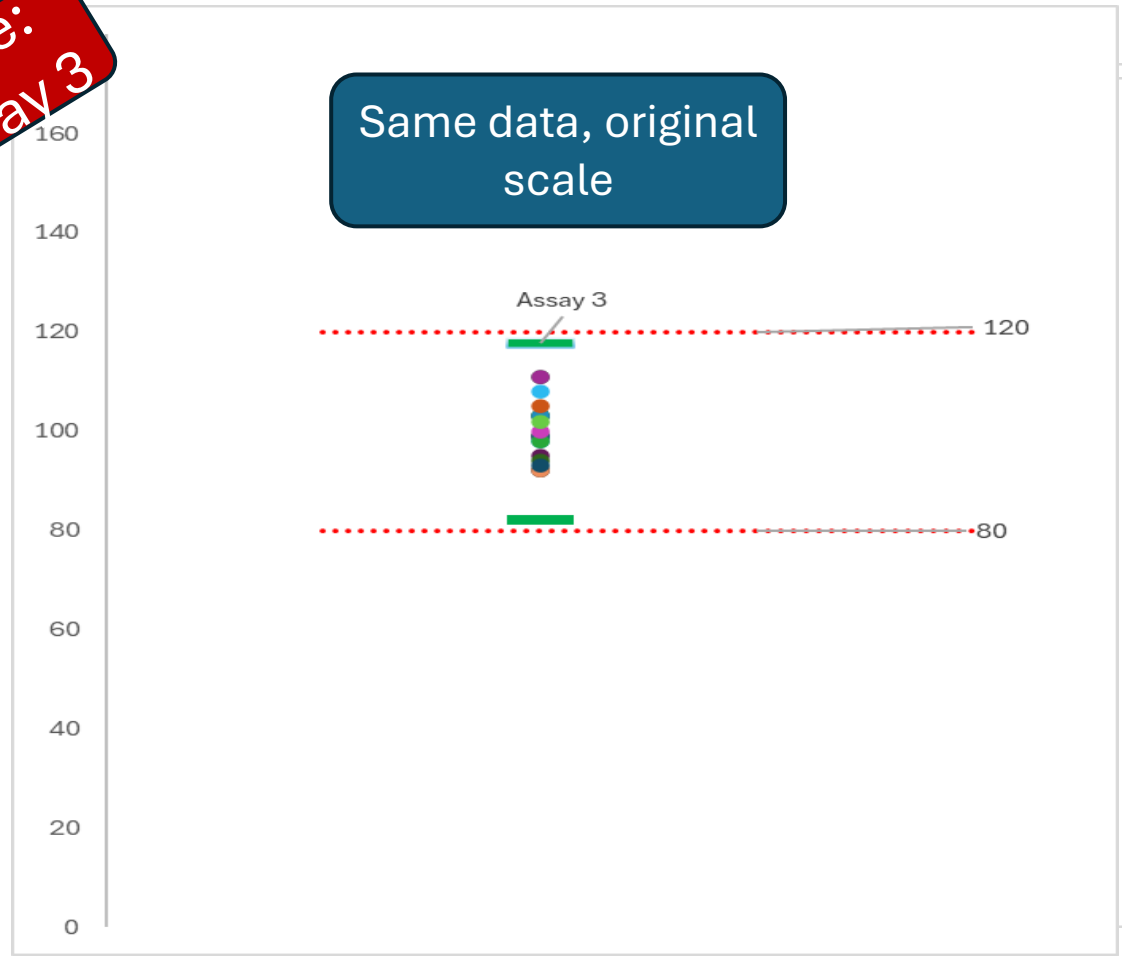
Specification Range is sometimes determined by the *inherent* "noise" in the assay

But, understanding the primary causes of variation in your bioassay system is essential: Often it is analyst capabilities or behavior of your cells (if a CBA)

Degradation and Stability Studies are gold mines for bioassay performance data, but proceed cautiously…

- Some molecules are more stable than others
  - *It can be difficult to demonstrate the bioassay is stability-indicating*
  - *Forced-deg may be needed, however, overly "forced" conditions can be irrelevant, misleading and not a good use of time*
- "Formal" stability conditions (temperature, light) are often useful to understand the stability-indicating nature of the bioassay
  - Plan for this with sufficient (i.e. additional) retain samples

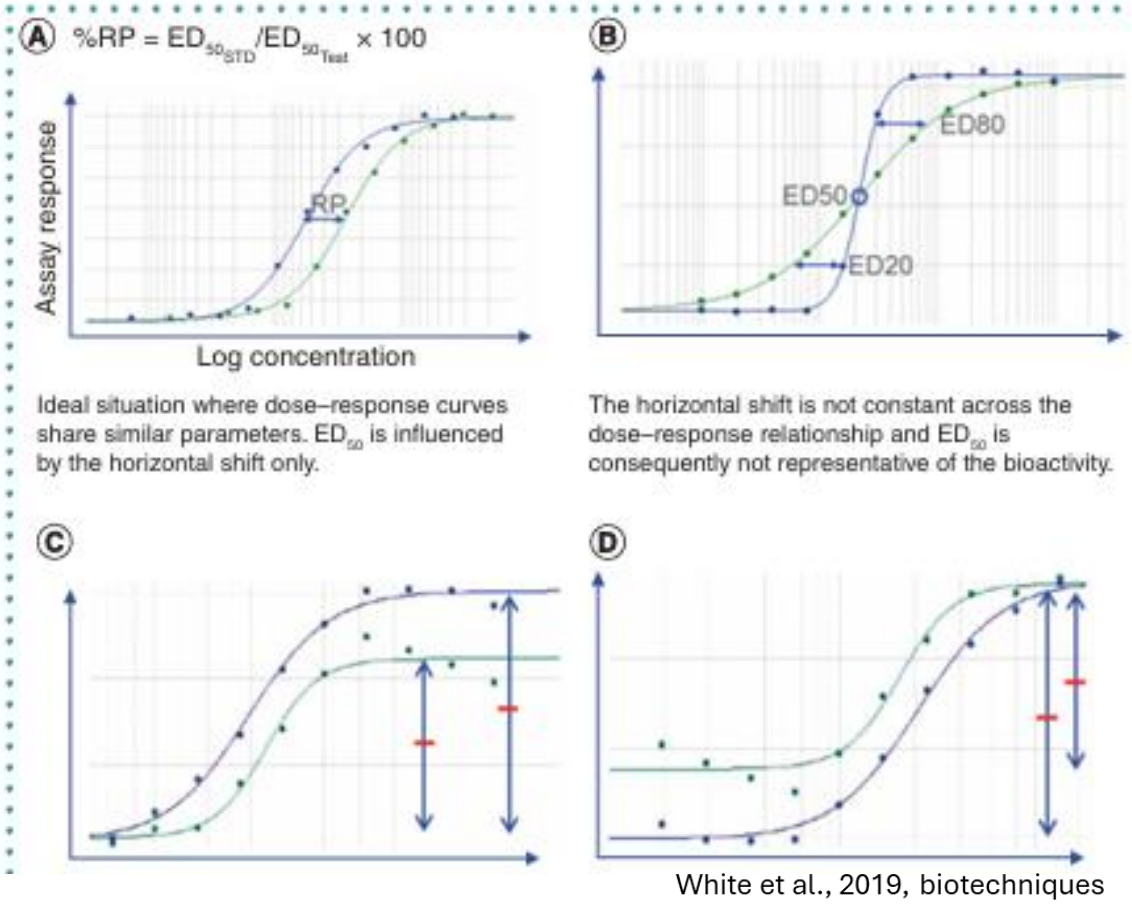
Case: Assay 3



TIP: Do not put bioassay in use for stability until its variability is well established

%RP values are only part of the evaluation – It’s not only about the numbers

- Does the sample behave like the reference in other ways?
  - Are the asymptotes similar?
  - Is the curve shape similar?



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Focus Areas for Long Term Success

- Reference Standards: Robust program to assure stability of all parameters and no significant changes or “drift” over storage condition.
  - *Make the largest possible amount – switching later will be painful*
  - *Track RS performance every time it is used – look for drift / change in many parameters*
  - *Replacement lots: Decide how to assign potency – prepare for debate ☺*
    - *Choose statistical criteria for “not different” – if achieved the burden of future management will be less. Test original vs. new with sufficient replication!*
- Release Specifications
  - *Also prepare for debate by learning how to explain RPA data*
  - *Engage company stakeholders, including commercial release lab*
  - *Review the different Health Authority guidelines, publications and notices!*
  - *Engage health authorities as early as possible / come with data!*
  - *Come to meetings like this – find out what others are doing!*

Not all therapies are the same (ADCs)

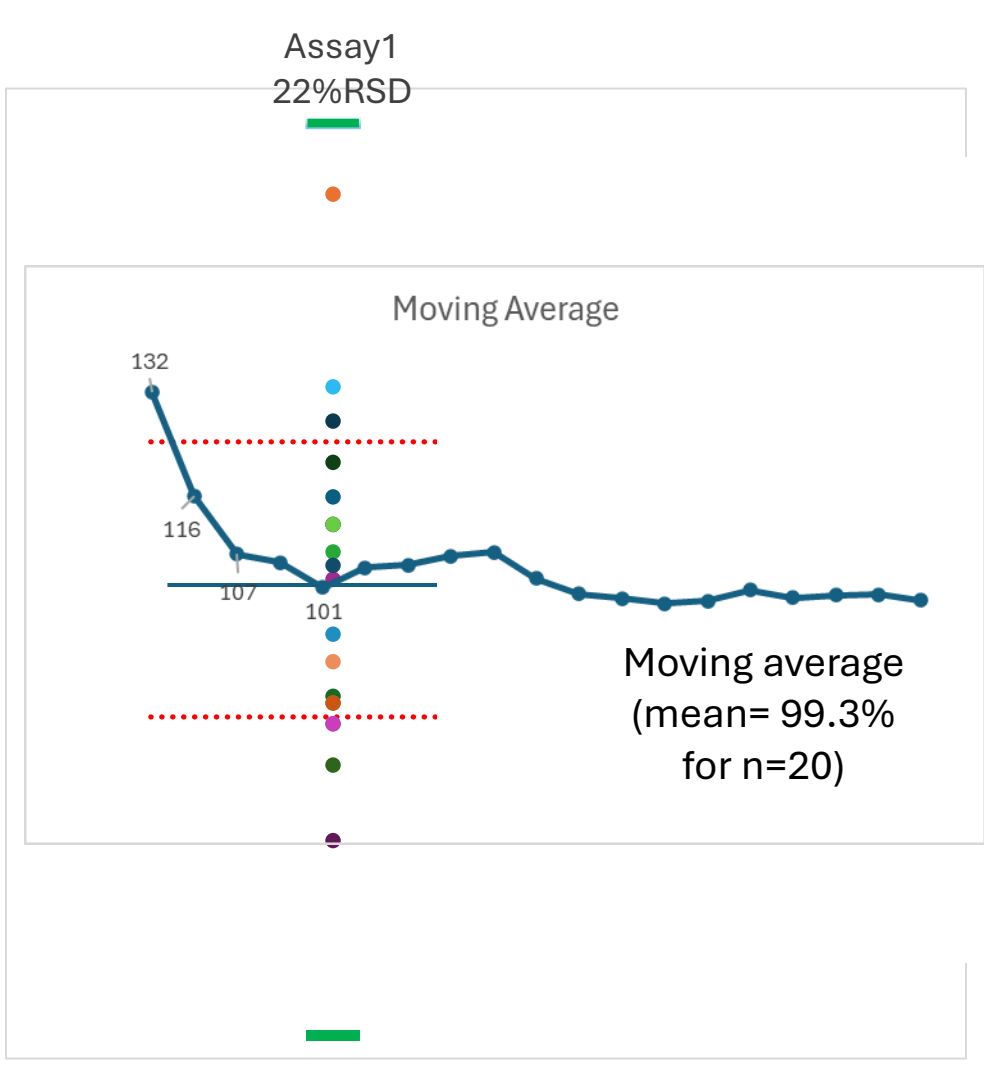
Successful bioassay organizations will…

- Design assay that meet country and & division-specific requirements
- Avoid “magic” solutions – understand “why”, test ideas scientifically
- Collect as much development experience as times allows
  - *Be methodical, collect the right kind of data! Develop test routines / training modules*
  - *Hire the right talent & assess properly = all can improve but some analysts are special*
  - *Within plate replication (n>2) is often NOT a major source of error and is often over-done and reduces assay throughput*
  - *A priori OOT/OOS procedures requiring additional testing are value-added for stability studies*
  - *If outlier testing is done, must be pro-active, well defined, and consistently applied*
    - *This is a hot-button topic with health authorities if not handled properly*
- Develop a close relationship with a dedicated statistician
  - *Make sure they understand your objectives and the science*
- Engage early with your commercial QC lab (or CMO)
  - *They may have special requirements (e.g. assay acceptance failure rates)*
- Avoid *un-necessary* OOT/OOS inspections by *unambiguous* assay acceptance criteria
  - *Rigid, consistently applied criteria that must be met before product result is calculated*
  - *Achieve consensus with your QA/QC/CMC regulatory team before issues arise*

As a last resort, if Inherent “Noise” is still too high (The Brute Force approach)

- Simply increase # of tests: the “average” is always closest to the truth
  - Lessens the risk of costly OOT/OOS investigations
  - But…. Higher effort/cost
- The number of assay replications needed can be calculated
  - e.g. n=4 may be enough in this case

A statistical approach can help here for study design

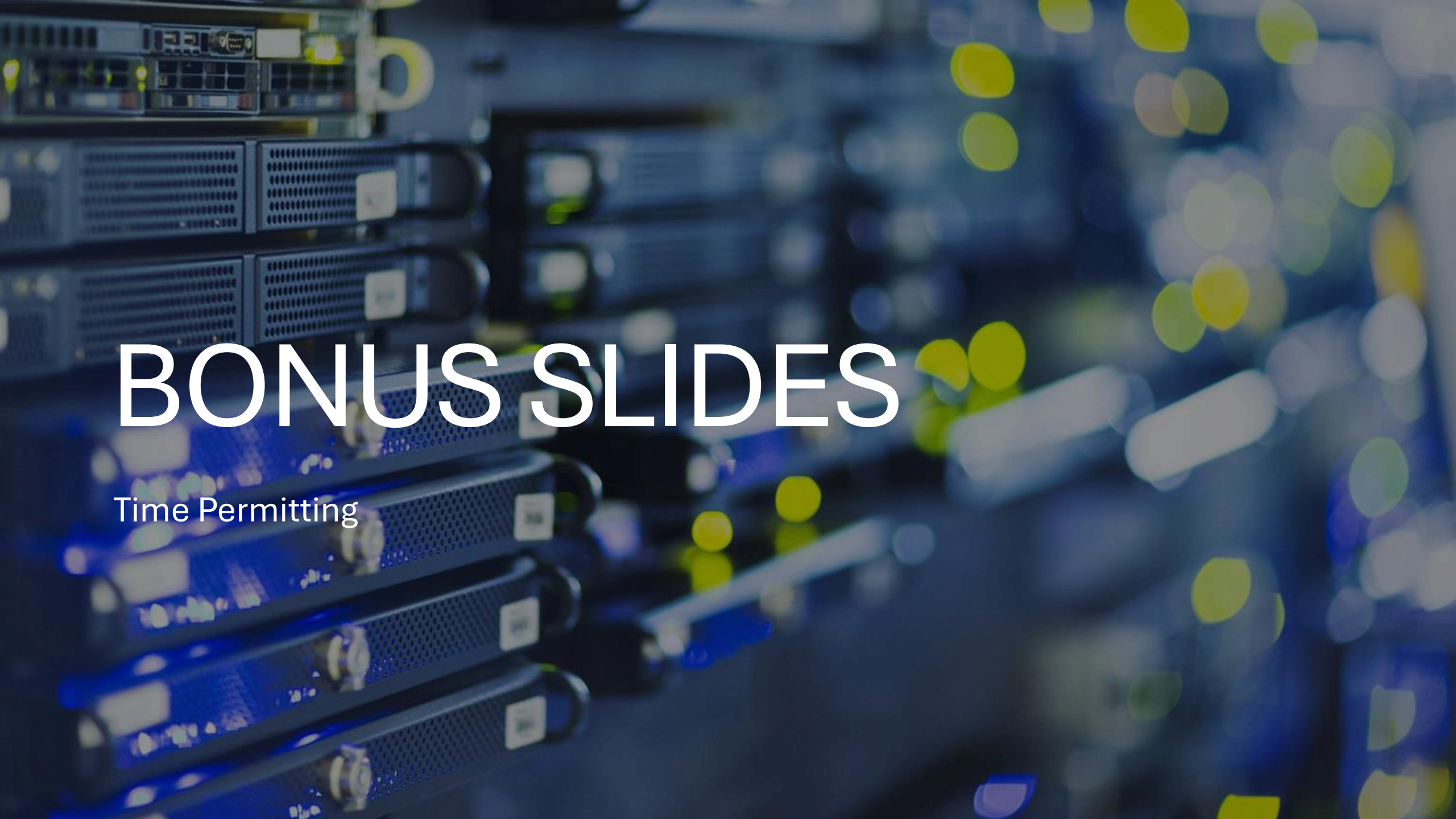


Lessons Learned / Key Points

- Expect to explain to project teams and management why bioassays add value and are needed – **become an advocate**
  - *Explain that In rare cases bioassay is the only method that can "see" manufacturing deviations or degradation processes*
  - *Articulate why health authorities require this test and historical context*
  - *Understand requirements for characterization vs. release*
- Work with your statistician to create a language explaining your specification limits – confidence intervals are often helpful
  - *Assure that assay noise has been minimized during development – i.e. is inherent or "as good as it can get"*
  - *This will also help in negotiations with health authorities*
- Pay attention / manage supplies of all materials
  - *Reference standards, control samples, reagents, plates, and all other materials that come in contact with the product during the bioassay*
- Create strategies for stability studies as early as possible
  - *Make your sample needs known, anticipate handling of apparent trends*
- Collect data to justify binding assays (where possible) as early as possible and before commercialization (get regulatory input)
- Stay current with new types of cells and improvements in technology – e.g. publication / these types of conferences!

Final Tip

Present / talk about what you do and explain results as often as possible- avoid jargon, simplify your explanations for non-experts!



BONUS SLIDES

Time Permitting

Comparing Assays or degraded samples

- Treatment needs to be incremental / gentle enough
  - E.g. temperature
  - *Very* Limited proteolysis – can be done quickly varying time of exposure to immobilized enzymes
  - Light – conditions hard to control, but can be appropriate

The simplest approach: When cell based assay shows (truly) lower activity, selectively test those samples by alternative method (e.g. ELISA) and build correlation database