Bioassay Evolution – Where Are we Now?

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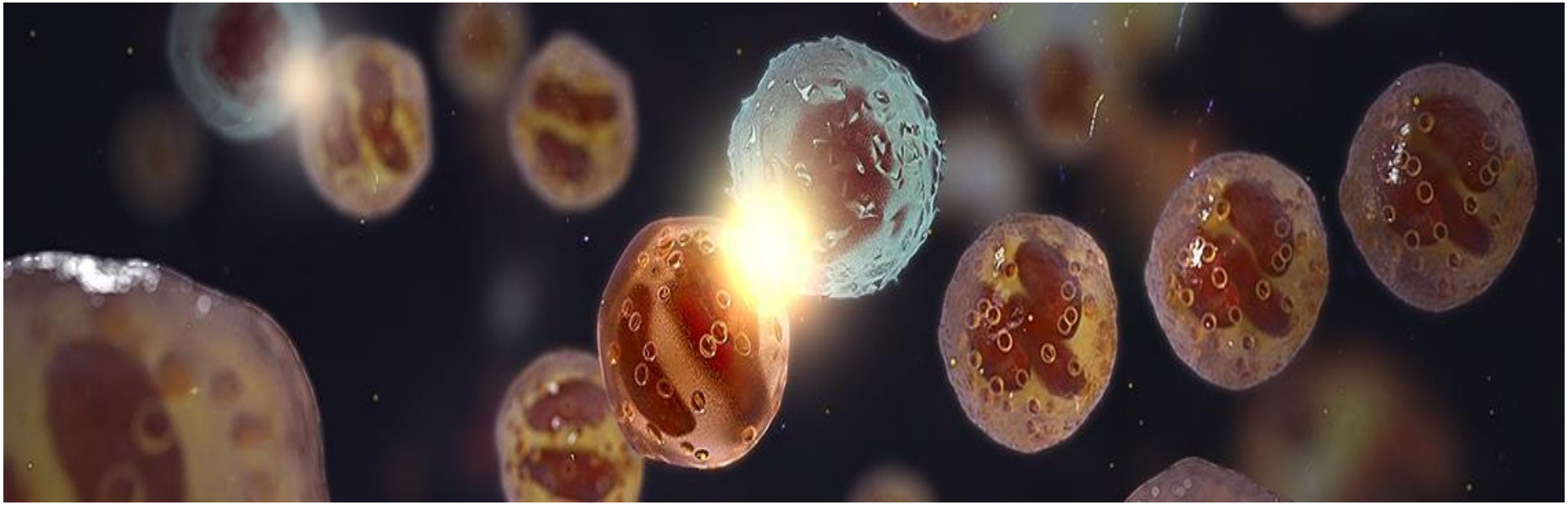


Why Are We Still Having Discussions On Bioassays?

- Bioassays were first developed in the late 1800's - you'd think we would know what to do by now
- However:
  - There is still no distinct regulatory guidance on expectations of what a bioassay should be
  - The way we analyze bioassays still gets questioned
  - We have new ideas on how to execute bioassays
    - Ready to plate cells, Mab categorization, QbD/DoE
  - Technology advances
    - Echo, New engineered lines, Multiplex immunoassay

Eternal Questions Often Remain

- What is a ‘bioassay’
  - Potency assay vs biological assay
- Why do we need a potency assay
- How close to a mimic of clinical efficacy does it need to be
- Which format to choose
- Reducing assay variability
- Calculating and reporting potency
- Changing from a cell based assay to receptor binding

Why Do We Have to Have A Potency Assay?

- Potency assays provide the only ‘functional’ test that tells us if the product is active
- Can be informative of changes to any aspect of product chemistry or structure, but ONLY IF THAT ASPECT PLAYS A ROLE IN ACTIVITY
- Only in vivo assays can illustrate differences in activity due to changes in PK/bioavailability
- Are essential in characterizing immunogenic responses – i.e. neutralizing capability of anti-drug antibodies

We Know What we Need From a Potency Assay

- It should serve a suitable purpose:
  - Biologically relevant
  - Stability indicating (preferable)
  - Appropriate precision
- It needs to be easy to run in a QC environment with low failure rates:
  - Simple format
  - Stable reagents such as cell lines
  - Includes proper controls
  - Robust
  - It must be validatable

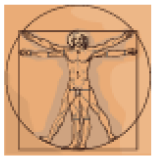
When Does a Potency Assay Stop Being a Biological Assay?

- In vivo assay
- Cell Based Longer Term Endpoint (e.g. Proliferation)
- Cell Based Short Term Endpoint (e.g. KIRA, Reporter Gene)
- Membrane Preparation Receptor Phosphorylation
- Cell Based Receptor Binding (e.g. FACS)
- Microtiter Based Receptor Binding
- Direct Ligand Binding (Mab)
- Physicochemical Tests

How Close to Clinical Efficacy Should the Potency Assay Be?

- ICH Specifications Q6B: ‘Mimicking the biological activity in the clinical situation is not always necessary’
- ‘A correlation between the expected clinical response and the activity in the in the biological assay should be established….’

The Bioassay Conundrum



Clinical study

In vivo models

Ex vivo models (e.g. whole blood assays)

In vitro assays based on primary cells

In vitro assays based on cell lines

In vitro cell-free assays (e.g. target binding)

Analytical methods addressing structural features

Elucidate MoA

Assessment of manufacturing consistency

- Batch release
- Characterization/ comparability

Direct relation to clinical effect

Technical QC suitability

Fredy Schnüriger, CASSS 2011

Once You Select Your Assay Type There is One Big Challenge to Get Over

There are Multiple Sources of Bioassay Variability and Ways to Deal with Them

- Cell Line Stability
- Dilution Effects
- Position Effects
- Plate to Plate Variability
- Assay Reagents
- Calibration of Equipment
- Operator
- Inter-laboratory Variation

Use of Automated Liquid Handling Systems an Excellent way to Control for Variability of Dilutions

Consistency

- Reduction in error caused by fatigue
- Eliminate bias introduced by analyst

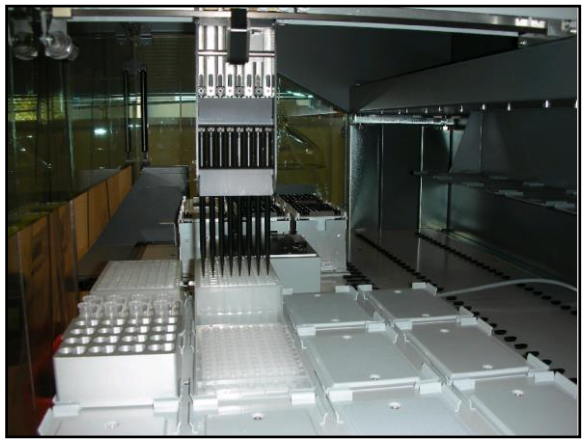
Accuracy

- Infinite ability to adjust actual volume delivered across diluents with varied viscosity
- Decreased potential for technical error

Safety

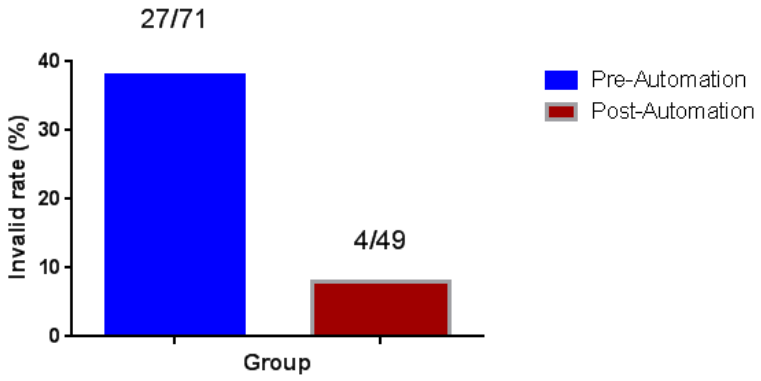
- Decreased potential for repetitive motion injury
- Increased throughput - one analyst can operate several robots simultaneously

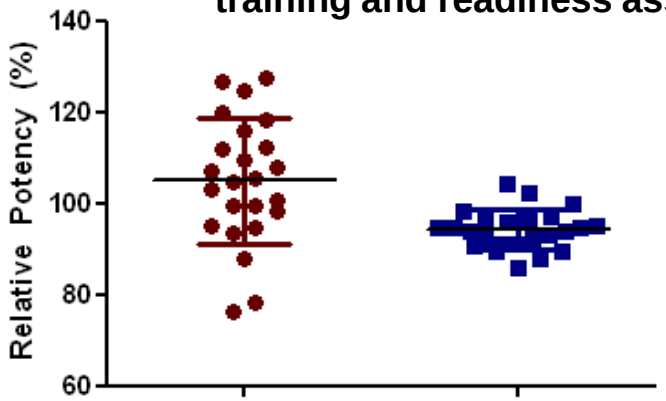
Automation alone will not “fix” a method which has not been optimized



Transferring lab had been running method routinely (approximately 10 assays/month) for 6 years. Receiving lab had only completed training and readiness assays

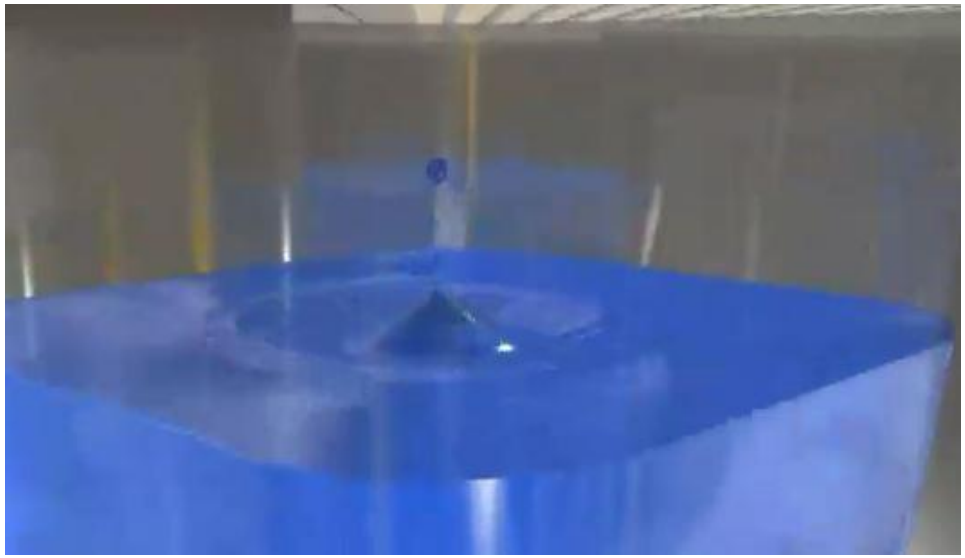
Reduced Invalid Rates

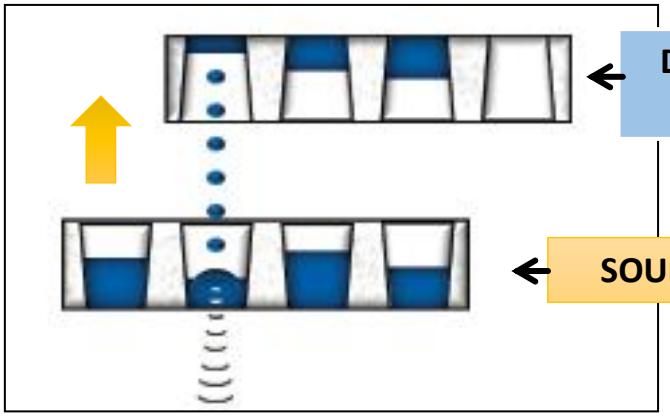


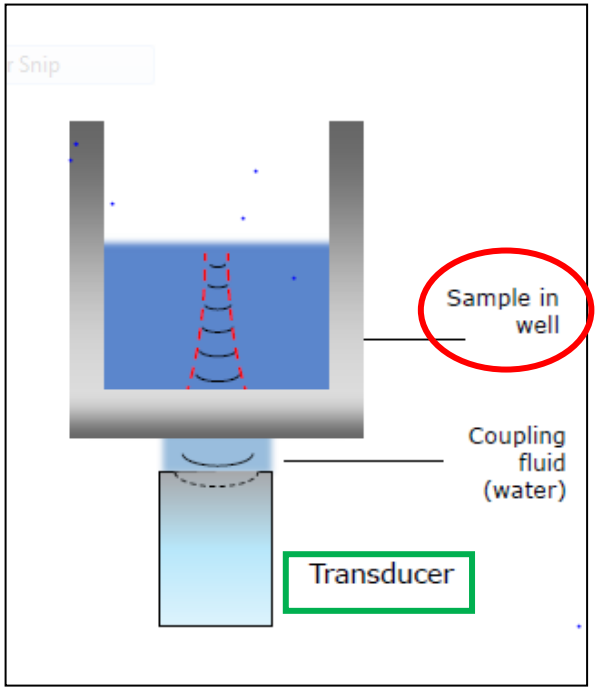


Labcyte Echo - Acoustic Droplet Ejection

An acoustic transducer returns to individual well. Determines amount of energy required to eject droplet(s)
The destination plate is upside down!







Images from Labcyte

Use of Frozen Cells can Reduce Variability and Eases the Burden of Growing Cells Continuously

Convenience and Flexibility

- Any assay could be set up at any time on any day of the week
  - Cells are never out of range

Consistency

- Same cell performance over time and across sites
  - Facilitate method transfers
  - Aid troubleshooting
  - Each bank qualified prior to use, therefore successful assays predictable.

Cost

- Continuous culture assures cells are always in ready supply, however most cells are discarded without use, thereby wasting resources and materials
- Business case for most bioassays estimated cost 1/7 of continuous culture if one site does all cell culture

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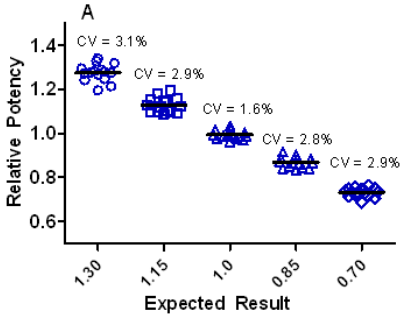
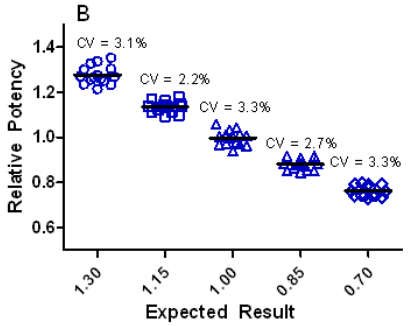
Use of Frozen Cells and Automation in Bioassays Has Reduced Assay Variability and Decreased the need for Multiple Plates

- Overall improvements, including Ready-to-Plate Cells and automation resulted in sufficient increase in precision to justify reducing number of assays required to report a result from 3 to 1. Method was re-validated to justify the change

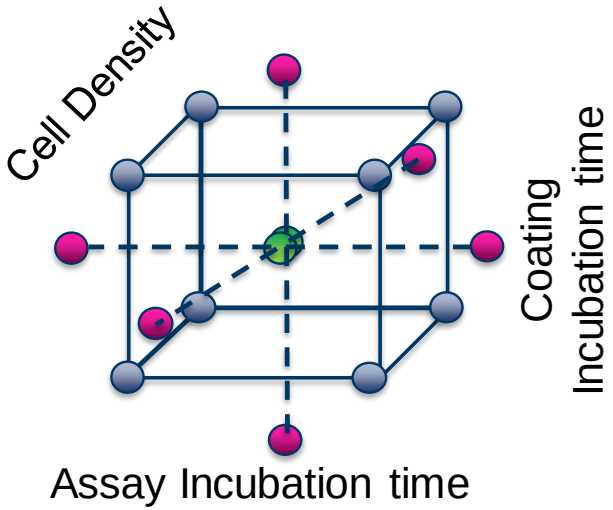
Overall %CV in 2006 validation = 6.4%



Overall %CV in 2009 validation = 3.0%

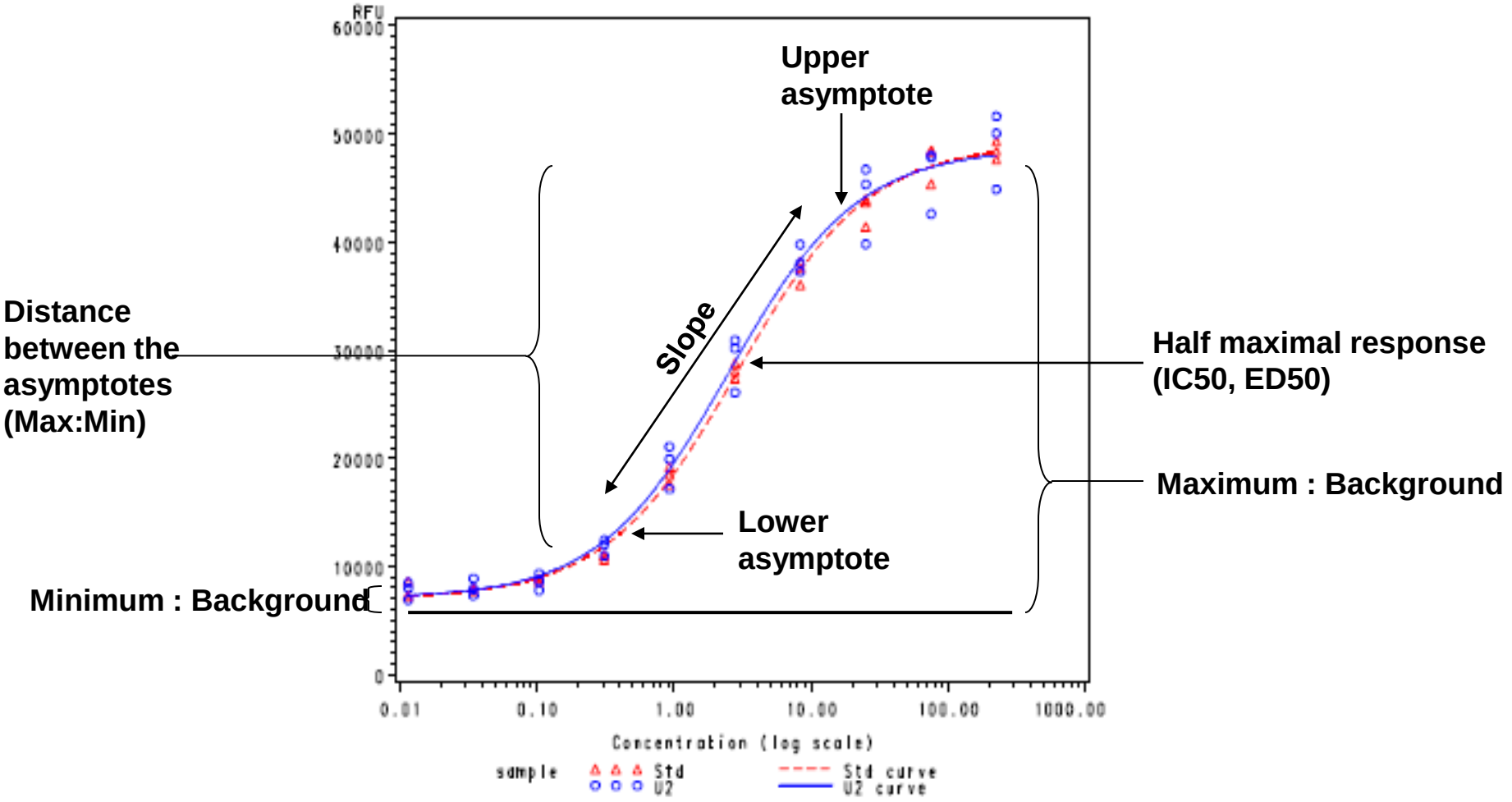


Using DOE to Explore Method Design Space



Block	Run	Cell Density	Assay Incubation Time	Caspase-Glo 3/7 Incubation Time
1	1	30000	28	60
1	2	45000	26	40
1	3	60000	24	60
1	4	30000	24	60
1	5	60000	28	40
1	6	45000	24	40
1	7	30000	24	20
1	8	45000	26	60
2	1	60000	28	60
2	2	45000	26	40
2	3	30000	26	40
2	4	60000	26	20
2	5	30000	28	20
2	6	45000	28	20
2	7	45000	26	40
2	8	60000	24	20

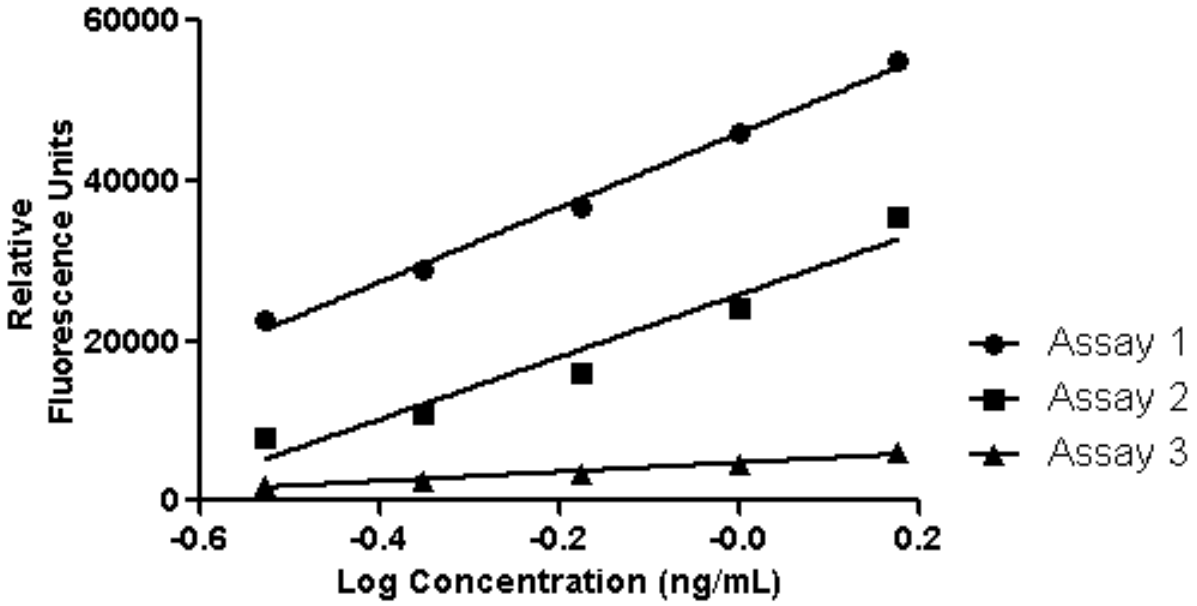
Agencies have not Approved Marketing Applications due to Lack of Appropriate Controls for the Bioassay



Determining which of these parameters to use, alone or in combination, can be a daunting task. Plus data transformation, curve fitting, weighting, masking, outlier detection and removal needs justification

Impact of Slope on Assay Accuracy and Precision

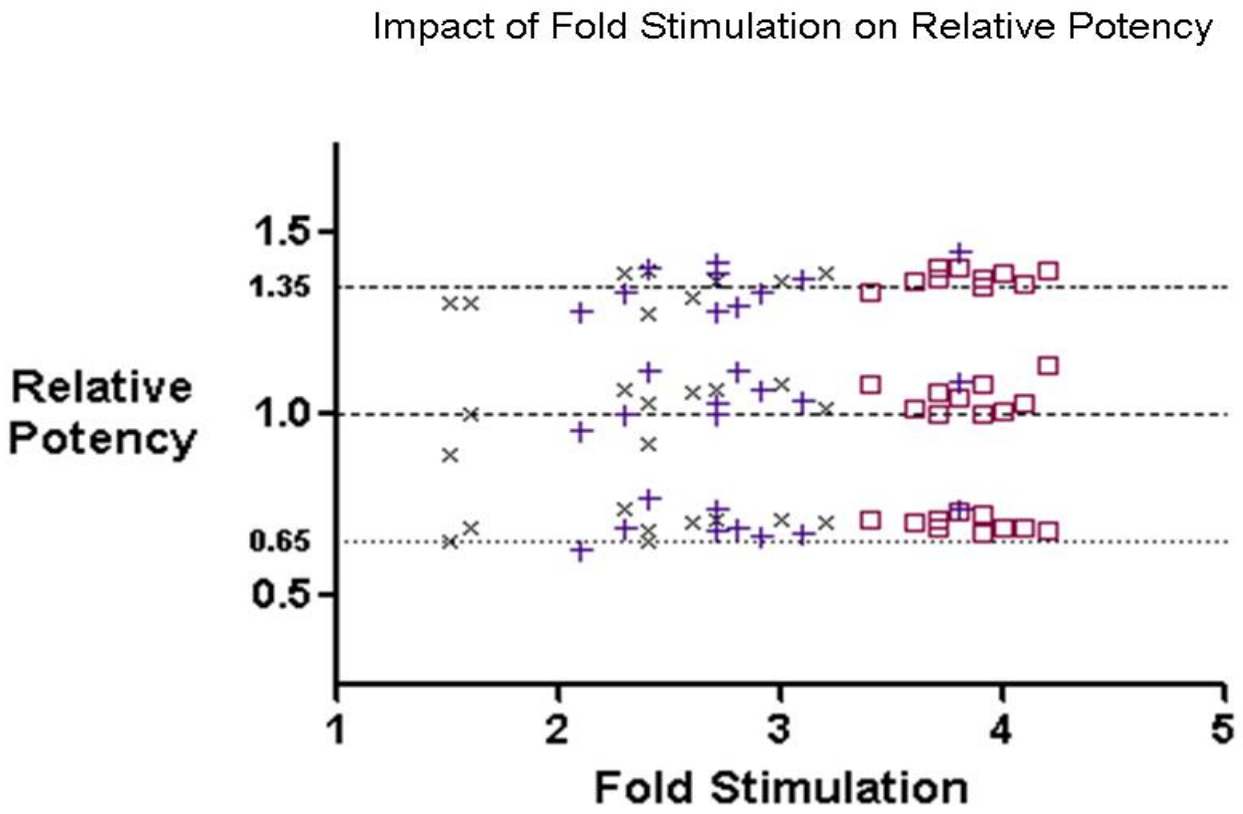
The graph depicts the reference standard response observed in three consecutive cell proliferation bioassays.



Two important questions are raised by these assays:

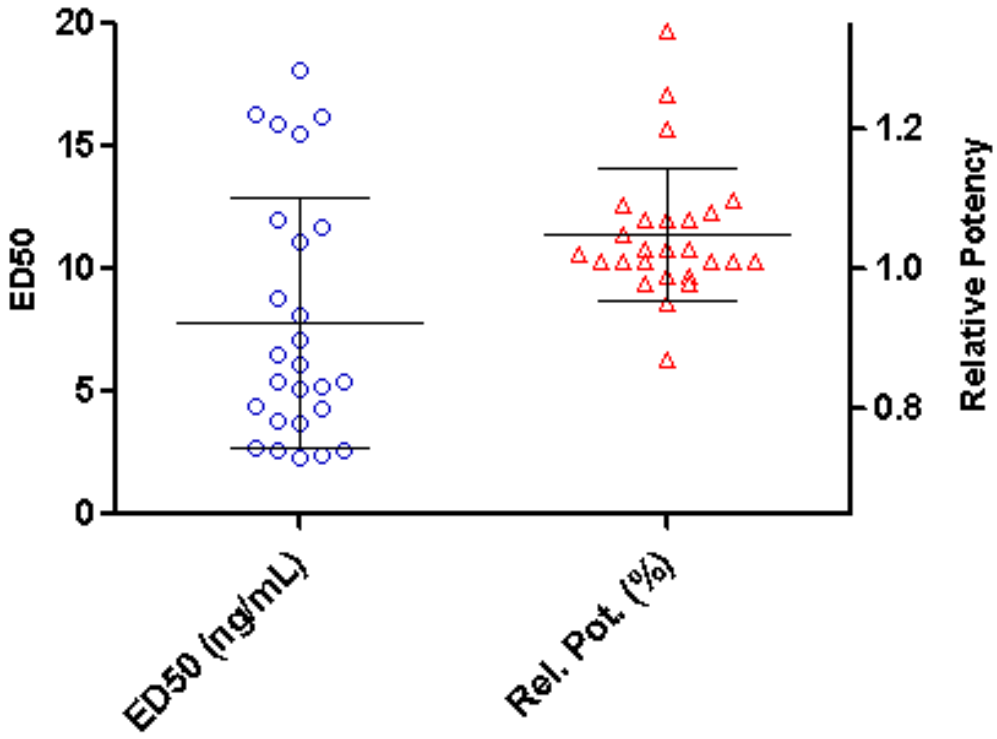
1. Should the lab be concerned about the difference in the absolute response of the reference standard?
2. If so, how does one determine the point at which assay capability is negatively affected?

This assay was accurate and precise at all response slopes observed



- All individual potency results were within 15% of the expected value, regardless of the corresponding fold stimulation
- The same results are obtained with both a 4PL or parallel line analysis

The variability of the ED50 response over time is much greater than that of a relative potency estimate



	ED50 (ng/mL)	Rel. Pot. (%)
Mean	7.82	1.05
Std. Dev.	5.09	0.09
% CV	65	9

- The ED50 results observed over the course of the 33 month trending period cover two thirds of the linear range of the method (1.56 – 25 ng/mL). Limits established using a 95% tolerance interval encompass the entire range (1.1 – 34.8 ng/mL).

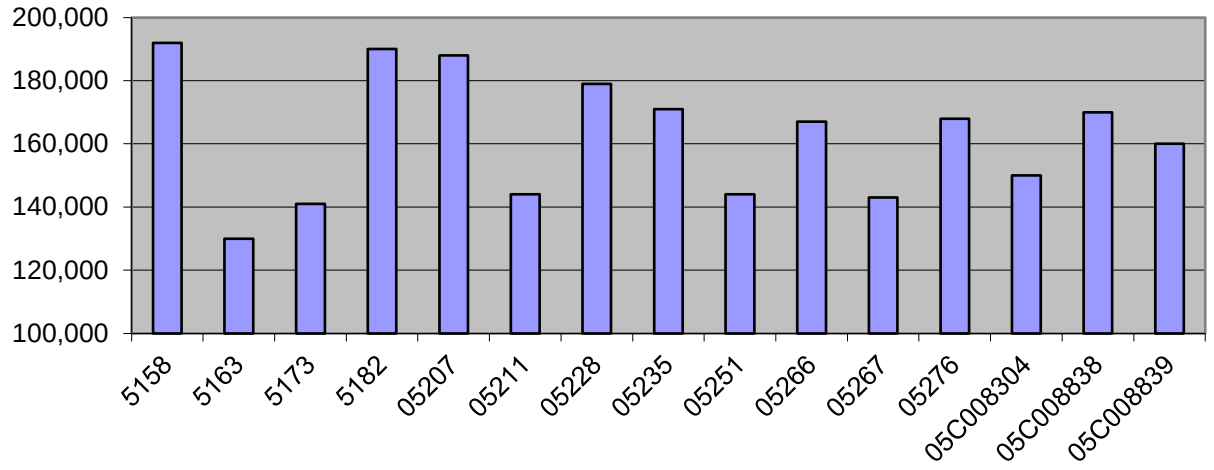
What Do You Need To Do To Replace Your Cell Based Bioassay?

- Show that normal and accelerated/stressed stability trends between binding and cell based bioassays are comparable
- Provide data that the binding assay can detect the potency of different molecular variants to the same extent as a cell based bioassay – use multiple types of material
- Have equal or better accuracy, precision and reproducibility
- For MAbs, if you want a Fab/ligand binding assay only, show that the Fc region of molecule plays no significant role in MoA/potency of antibody
    - Glycosylation of the antibody plays no significant role in potency
    - Complement plays no significant role in potency
- Understand if the molar equivalent of Fab has comparable
    - Binding to ligand
    - Ligand neutralizing ability

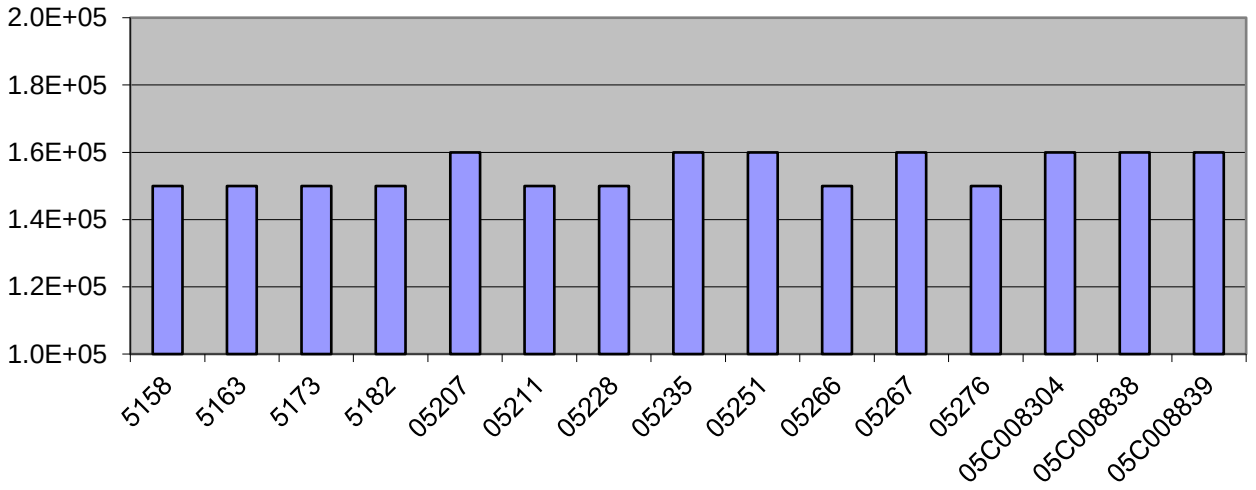
Batch-to-Batch Variability: Fact or Fiction?

10-years innovator experience shows no variability in potency

Lot Release Data



Single Assay

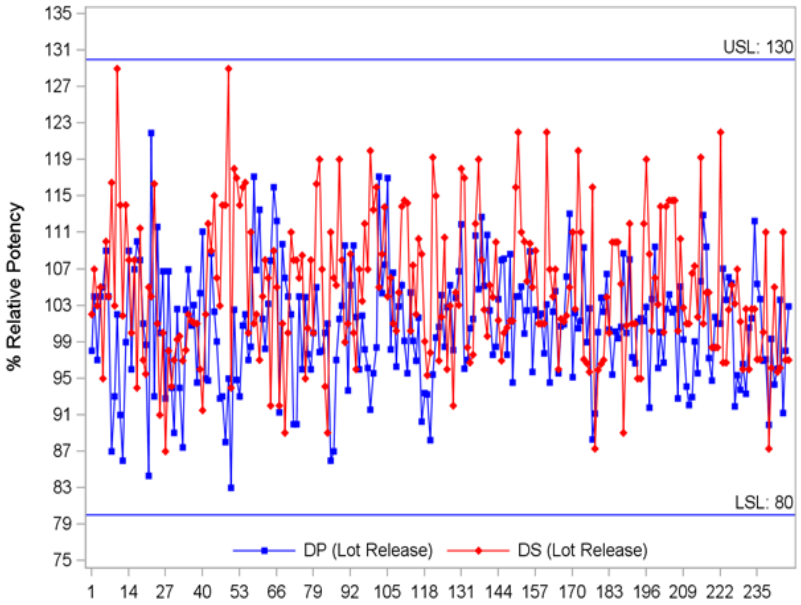


Product Potency Testing can be Retained on the DS Specification and Removed from DP Specification or Visa Versa

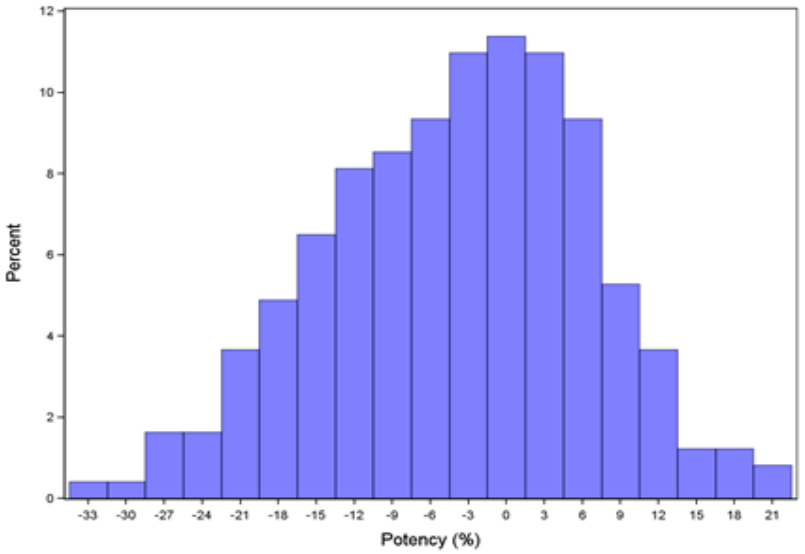
Summary of Testing Assessment

- No significant change in product potency between DS and DP under normal processing conditions
- Characterization studies demonstrate that DP process stresses are not expected to impact potency, critical operating parameters are well controlled
- Aggregation is the primary degradation product impacting potency. Changes would be detected by SE-HPLC which will be retained on the DP specification

Overlay Plot of DS and DP Potency

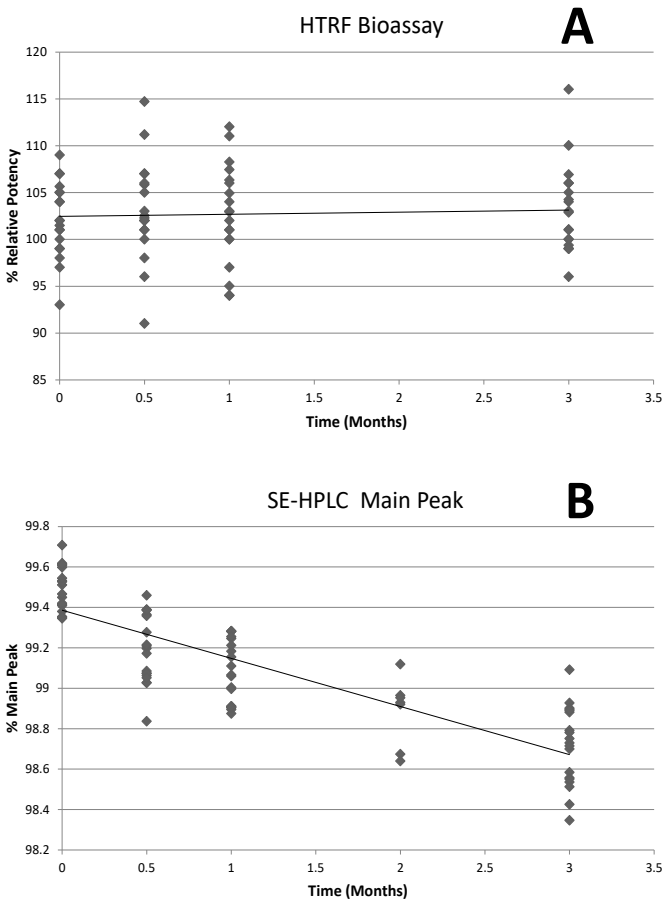


Histogram of DS and DP Potency Paired Differences

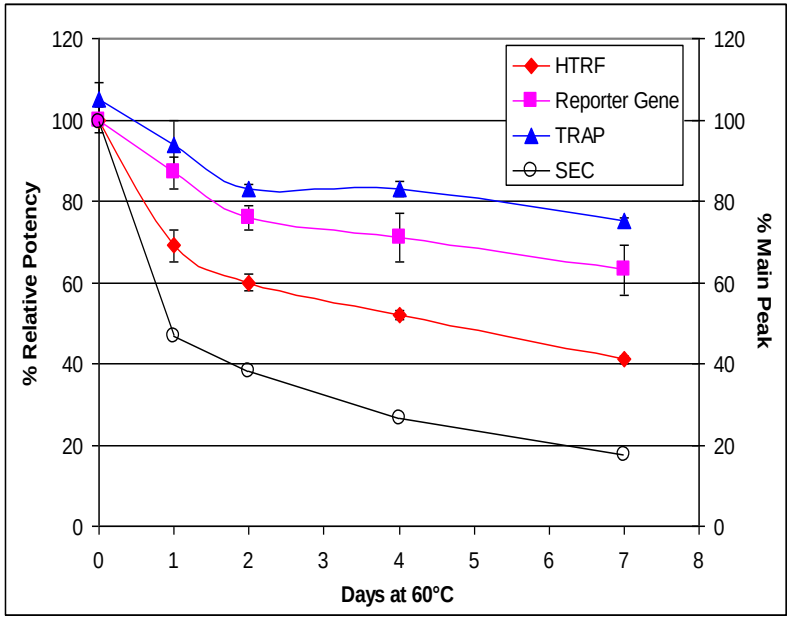


Aggregation is Often the Primary Degradation Product with the Potential to Impact Potency. Changes can be more Readily Detected by SE-HPLC

Impact of Accelerated Thermal Storage Condition at 37°C of mAb X Assessed by HTRF Assays (Panel A) and SE-HPLC (Panel B)



Impact of Accelerated Condition at 60°C of mAb X Assessed by SE-HPLC and Bioassays



Orthogonal methods which control for product-related species that are known to impact potency will remain on the specification

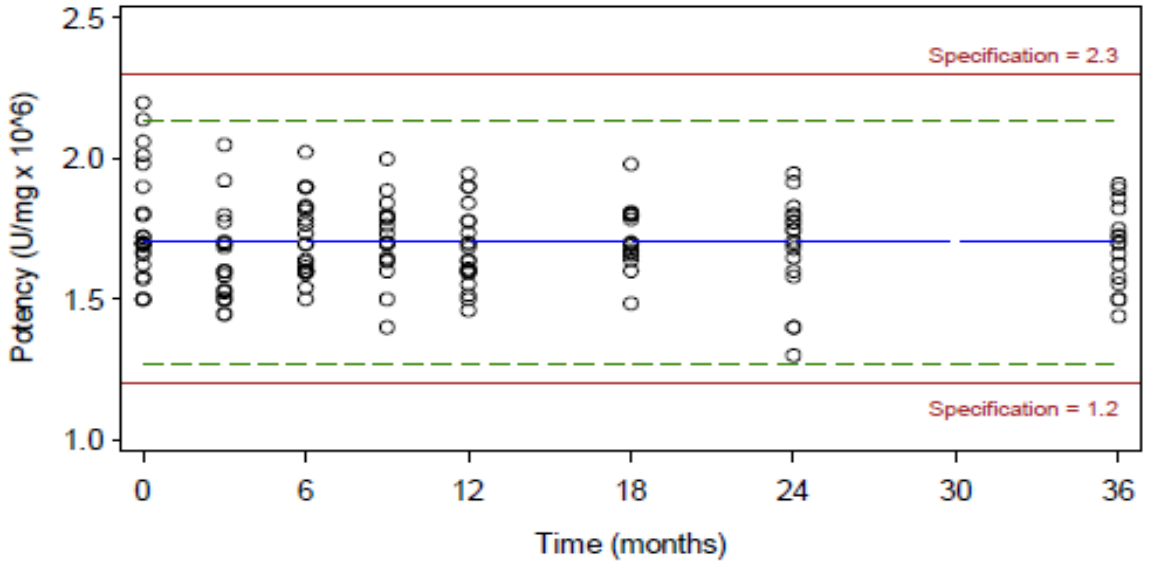
- Mis-folded and high-molecular weight species, both of which negatively impact *in-vitro* potency, are controlled by the HIC and SE-HPLC methods, respectively.
- Consistency of the DS release data demonstrating no OOS results through the history of DS manufacture
- Potency will be measured on either Drug Product or Drug Substance

Presence of SE-HPLC and HIC on the specification which control for species known to impact potency and can serve as a surrogate measure of potency at this stage of manufacture provide assurance of supply

Frozen Drug Substance does not Exhibit Potency Reduction over time, so can be justified to Remove Potency from DS Stability Studies

Frozen State Degradation Pathways

Molecular mobility is very restricted in the frozen state. Therefore physical and most chemical modifications typically do not occur



No change in potency after three years for multiple lots

Conclusions

- Biological assays are the only assays that can directly measure the functional, biological activity of biological therapeutics
- Selecting a potency assay for lot release may involve validation against a more ‘clinically relevant’ assay during development (from cell line to receptor binding)
- The appropriate design of bioassays is important if the results they provide are to be valid and useful. Attempts must be made to assess and reduce variability and bias

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