Validation/Qualification Issues for Enabling Technologies for Drug Discovery

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BD Biosciences

Who is BD?

- **FORTUNE 500** company (#316)
- Locations in **more than 50 countries**
- Approximately **29,000 associates** worldwide
- Serves healthcare institutions, life science researchers, clinical laboratories and the general public
- Sells a broad range of medical supplies and services, devices, laboratory equipment and diagnostic products
- BD Gentest^{SM} Contract Research Services provides *in vitro* drug metabolism services

My goals today

- Bring a CRO industry perspective on assay validation
- Offer a view on challenges to successful validation
- Review a case study from our laboratory
- Recommendations and closing thoughts

What is Validation?

- Validation is demonstrating you can repeatedly do what you want to do.
  - That means knowing how your inputs relate to your outputs
  - It starts early in the process/product development and continues throughout commercial life of the product.
- Must be fit for purpose
  - Simple QC assays for a research use only product
  - Safety study assay that is correlated (or predictive) of a clinical outcome.

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CRO Perspective

- Not significantly different than any other life science business
- We must meet expectations of:
  - Regulatory agencies
  - Customers (funding)
  - Auditors (agencies, customers, consultants)
- Our customer base
  - Mostly large and small pharma
  - They require “research grade” to GLP standard assays
  - Customer philosophies vary – as a service provider we need to achieve a validation standard representative of the highest standard among our target customer base

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Challenges to Assay Validation

- It is resource intensive
- Requires multi-disciplinary expertise
  - Scientists
  - Statisticians
  - Quality Assurance
  - Project managers
- Relative to the science and other end goals, the process can be dull and tedious
- Communication - keeping all project team members aligned and stakeholders informed

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General Strategy for Assay Validation

Qualitative requirements

- Prework
  - Lock in on project goals
    - Input from various sources [Regulatory guidelines, “Voice of customer”, Direct study of customer environment (e.g. scientific literature, etc)]

Quantitative requirements

- Verification
  - Conduct the needed experiments to become adequately familiar with the assay conduct, QCs, reproducibility and robustness
  - Adequate verification data sets make the validation exercise much easier from a quantitative and statistical perspective.
  - With robust data going in, validation should be a coronation.
- Validation
  - Demonstration and documentation that acceptance criteria can be met
  - Make recommendations for standard assay conditions

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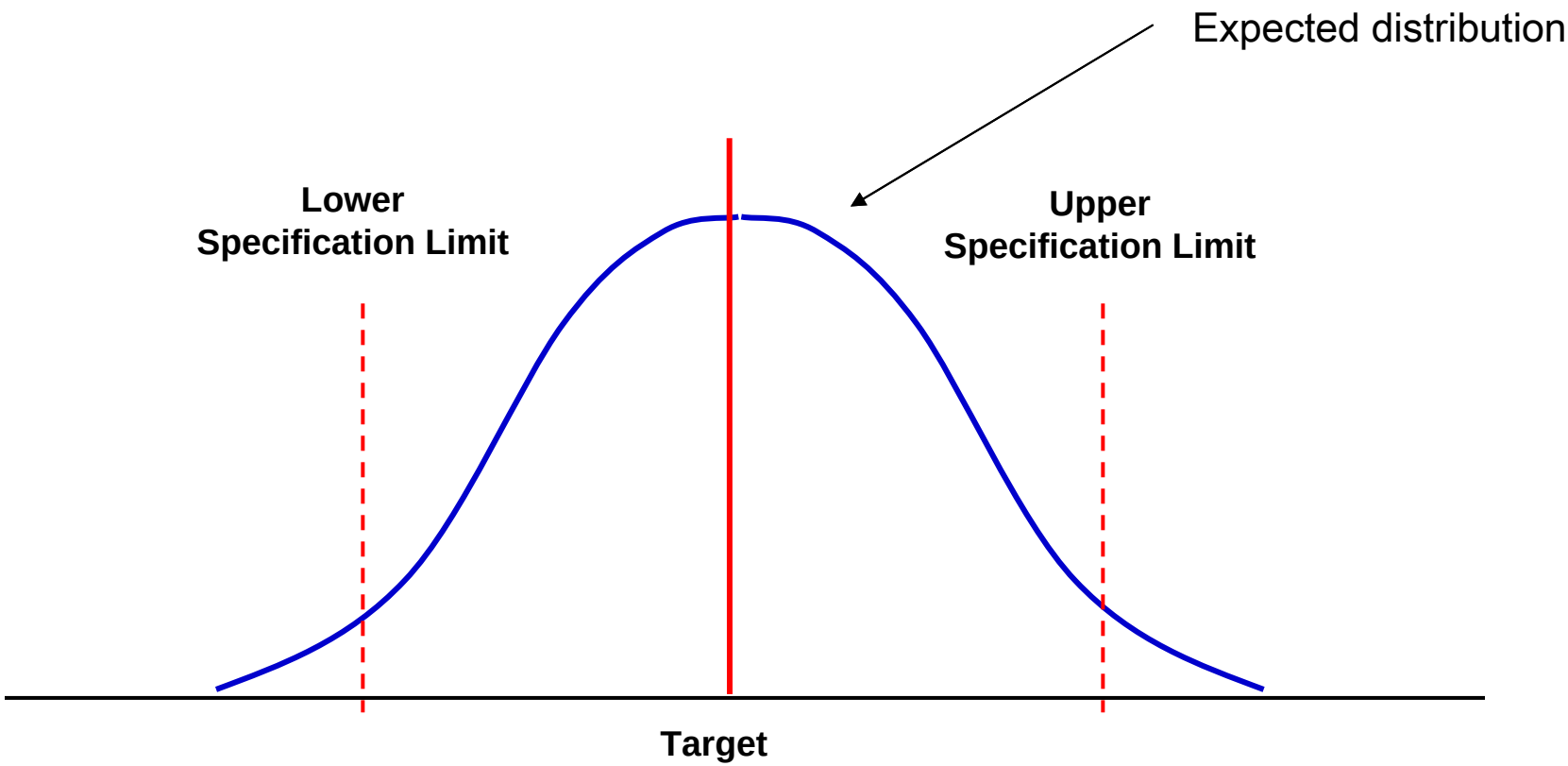
Validation Components

- Intra-assay precision (i.e. multiple replicates of the same conditions are tested in one assay)
- Inter-assay precision (i.e. repeat assays conducted by the same operator). If the repeat assays are conducted on separate days, this test may also be referred to as inter-day precision.
- Inter-operator precision (i.e. identical assays conducted by different operators either side by side or sequentially as applicable)

Validation Components

- Acceptance criteria
  - The validation protocol must specify acceptance criteria for all parameters to be validated.
  - Acceptance criteria are determined by the Study Director (with input from stake holders if applicable).
  - Acceptance criteria may be based on a number of considerations, including, but not limited to
    - Historical in-house data
    - Results of verification experiments
    - Voice of customer
    - Industry standards
    - Competitive environment
    - Regulatory guidelines
    - Scientific literature

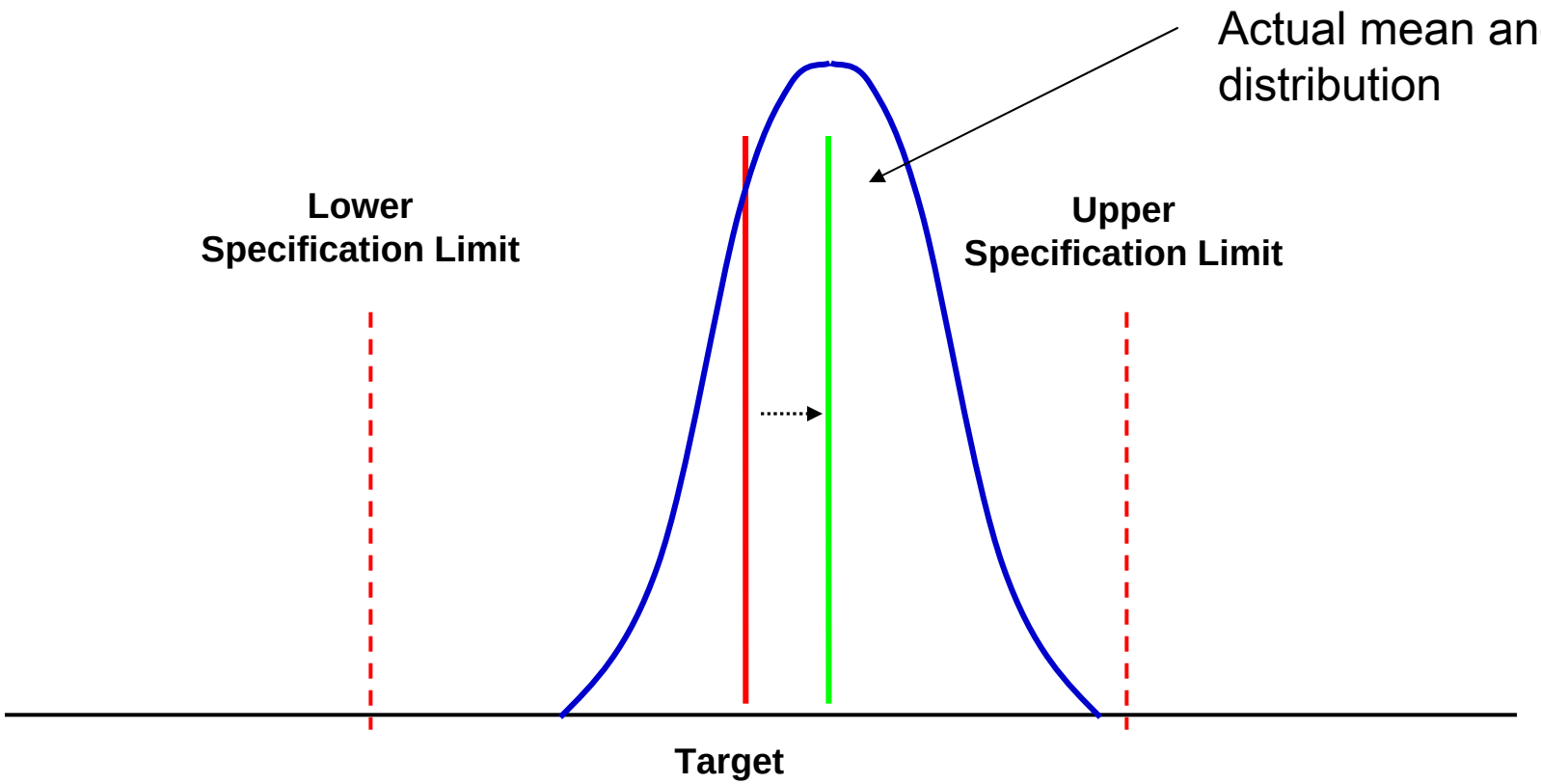
Validation Components



- The number of QC parameters and statistical precision will dictate failure rate. For example, 4 QC values at 95% equals a 20% failure rate
- Focus on the right number of QCs and the right level of statistical failures.
- The "flip side" of broad statistical criteria is the acceptable range may be excessively large

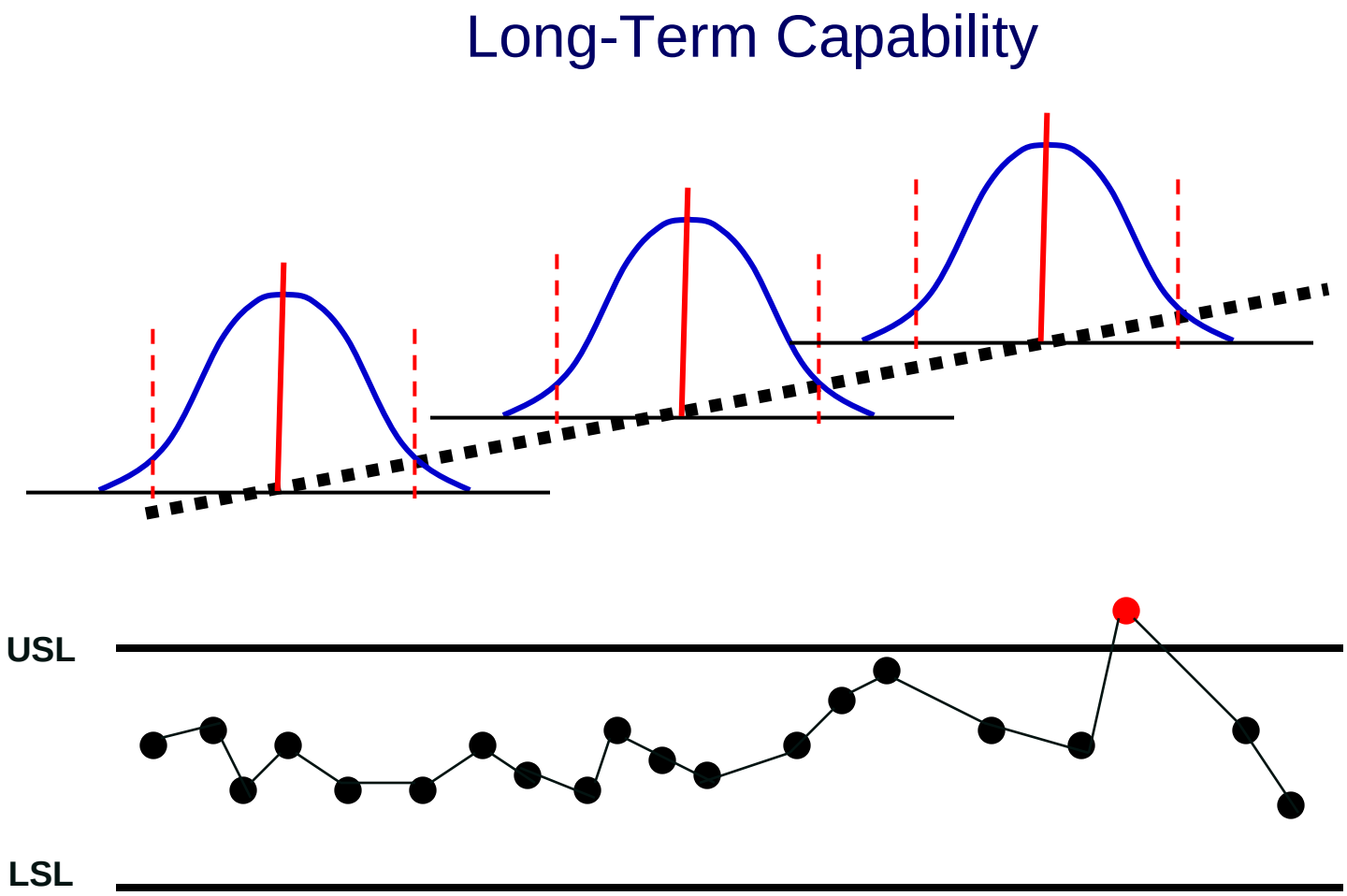
Validation Components

With data accumulation over time…



- The limits may evolve over time as more data accumulates
- Other driving factors for modifying limits includes changes in customer expectations, waste elimination targets, etc

Validation Components



- Is an upward/downward trend observed? Examine reasons and take corrective action if possible

Assay “Qualification”, not validation

- Demonstrate reproducibility only
  - Typically interday
- Establish a positive and negative control
- “Research grade”
  - No formal protocols, reports
  - No formal involvement of QA
- For many customers, this meets their expectations

Structured process to validation

- Establish a process with check points to promote care and proper planning (e.g. peer review, QA review)
- Check points represent “control” points in the process

Case Study - Validation

- Project Objective Statement: Adapt cytochrome P450 inhibition assays to mass spectrometry analytical methodology and introduce preincubation to standard protocol.
- Cytochrome P450 inhibition is a required drug-drug interaction test for small molecule drug candidates
- Analytical method validation
  - 8 metabolites; 8 validation protocols
- Assay method validation
  - 8 assays; 16 Validation protocols for IC_{50}, K_i, time-dependent inhibition analysis
- 48 Validation protocols and Reports

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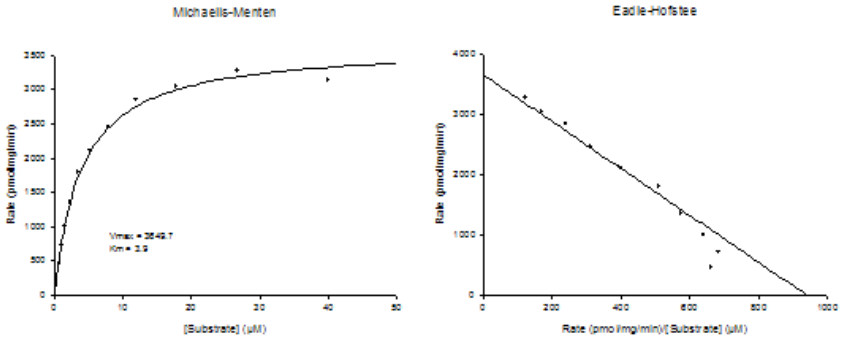
Acceptance Criteria

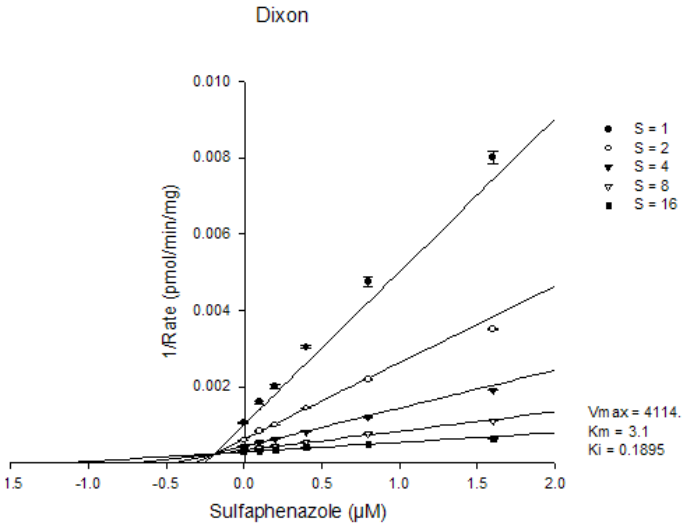
- Analytical
- FDA guidance document for analytical method validation (2001)
  - Selectivity
  - Standard Curve
  - Stability
    - Autosampler
    - Freeze/thaw
    - 4 weeks @ - 20 ºC
  - Accuracy and Precision

- Assays
- Incubation time(s) selected must fall within linear portion of the response
- Protein concentration(s) selected must fall within linear portion of the response
- Total metabolism must be less than 15%.
- K_M value must be within 5-fold of literature values reported by Obach and Walsky, Drug Metab. Dispos. 32: 647, 2004.
- IC_{50} values must be < X µM and duplicate determinations within 5-fold
- K_i values must be within 5-fold of the IC_{50} value and less than twice the IC_{50} value. Duplicate determinations within 5-fold

Example Assay Development and reproducibility: CYP2C9/Diclofenac 4'-hydroxylase

- Resulting Data Set
  - Linearity of metabolite formation with incubation time and HLM protein concentration
  - K_M determination
    - 3.5 µM, 3.9 µM
  - IC_{50} and K_i determination with sulfaphenazole
    - IC50: 0.41 µM, 0.63 µM
    - K_i: 0.20 µM, 0.19 µM





Assay Validation Results

Parameter	Criteria	Results
Time dependence	Incubation time(s) selected fall within the linear portion of the assay	**Pass** (5 min)
Protein Dependence	Protein concentration(s) selected fall within the linear portion of the assay	**Pass** (0.02 mg/mL)
Total metabolism	Less than 15%. If assay sensitivity is a problem the study director will determine if up to 30% total metabolism is acceptable.	**Pass** (7% at 0.25 µM midazolam, 0.04 mg/mL protein, 5 min)
K_M	Within 5-fold of literature values reported by Stresser et al; Drug Metab. Dispos. 32: 105-112, 2004 (3.0 µM) or as determined by Obach and Walsky, Drug Metab. Dispos. 32: 647-660, 2004 (2.3 µM).	**Pass** (2.0 µM, 2.3 µM)
IC_{50}	Ketoconazole: < 1 µM; Duplicate determinations within 5-fold	**Pass** (0.013 µM, 0.019 µM)
K_i	Within 10-fold of the IC_{50} value and less than twice the IC_{50} value; Duplicate determinations within 5-fold	**Pass** (0.0086 µM, 0.0092 µM)

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Assay Validation Results (cont)

Parameter	Criteria	Results
IC_{50} shift	The shift in IC_{50} for azamulin, verapamil, and diltiazem should be > 2-fold at the 30 min preincubation time point; the shift in IC50 for ketoconazole should be < 2-fold	**Pass** Ketoconazole (0.9, 1.0) Azamulin (76, 44) Verapamil (62, 97) Diltiazem (>33, >26)
K_I	Within 5-fold of the literature value reported in Obach et al (2006) for verapamil (1.8 µM) and diltiazem (4.5 µM). Within 10-fold of the mean value obtained during feasibility experiments for azamulin (0.17 µM). Duplicate determinations within 5-fold of each other.	**Pass** Azamulin (0.10 µM, 0.23 µM) Verapamil (1.6 µM, 2.4 µM) Diltiazem (13 µM, 4.4 µM)
k_{inact}	Within 5-fold of the literature value reported in Obach et al (2006) for verapamil (0.043 min^{-1}) and diltiazem (0.012 min^{-1}). Within 10-fold of the mean value obtained during feasibility experiments for azamulin (0.50 min^{-1}). Duplicate determinations within 5-fold of each other.	**Pass** Azamulin (0.54 min^{-1}, 0.82 min^{-1}) Verapamil (0.023 min^{-1}, 0.022 min^{-1}) Diltiazem (0.0024 min^{-1}, 0.0076 min^{-1})

Outcome and observations

- All validations met their acceptance criteria
- Not always “smooth sailing”
- Amended protocols or protocol deviations
- Amendments and deviations should be avoided
  - Unexpected time and effort to discuss, resolve & document
  - Frustration to project teams
  - Represent obstacles to successful validation

Example deviations

1. Organic solvent used by analytical chemist was slightly different than that used by the assay biochemist (2.5% vs 0.3%). Eventually required amended protocol to demonstrate lack of an effect.
2. Protocol created unattainable mandate
   - "IC_{50} value will be reported" (was greater than highest concentration tested – no effect on conclusion)
3. Unanticipated results during validation experiment caused a change in substrate concentration
4. Analyst forgot a (non-critical) step
5. Instrument malfunction meant exceeding the stability time point specified in the protocol.

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Tips to avoid deviations

- Ensure analysts understand the task and are aware of what could go wrong. Don’t assume.
- Incorporate specificity into the protocol to provide guidance, but adequate flexibility to avoid painting yourself into a corner
- Don’t skimp on time needed for verification

Closing thoughts

- It is unlikely you will have all the information and forethought needed to avoid deviations, amendments and other “issues”
  - Resolution can range from simple to down right “painful”. Input from key stakeholders, “voices of reason” and experienced individuals result in best outcomes.
- With cell-based assays, variability is larger than for biochemical endpoints described here. Long term drift is more of a concern.
- An ounce of planning is worth a pound of reactive effort

Thank you for your attention

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