Hindsight is 20/20!
50 Combined Years of Bioassay Experiences

Chana Fuchs, Ph.D.

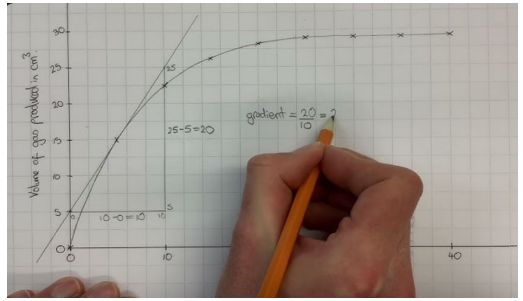
Retired FDA
Biologics CMC Reviewer

Nadine Ritter, Ph.D.

Global Biotech Experts
Biologics CMC Consultant

Pop quiz at the end! *Hint: Neither of these amazing scientists are us…*

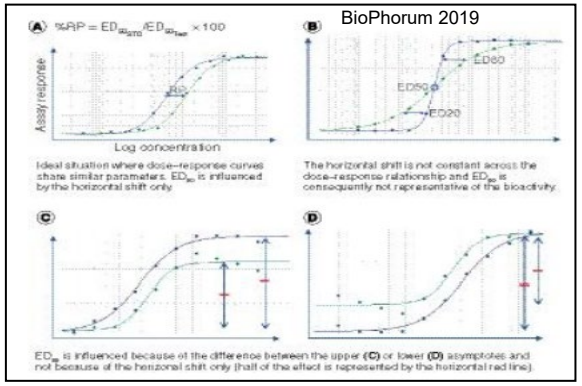
Potency Bioassays for Biological Products: Then vs Now



THEN (1900's – 1970's)

- Biological products were naturally derived (insulin, growth hormones, blood factors, live attenuated vaccines)
- Potency was mostly tested with in vivo bioassays (eg for hormones, vaccines) with minimal replication scheme (expense and ethics)
- In vitro bioassays (eg for enzymes) used crude, highly variable reagents
- In vitro reactions used simple analytical instrumentation (eg colorimeters)
- In vitro bioassays used large volume reactions (no adjustable micropipettes, no microfuge tubes, no 96 well plates)
- Glass pipettes and reaction tubes had to be washed (occasional soap or acid carryover)
- Challenging temperature control during incubations (positional and temporal biases in water baths)
- Manual measurements (one tube at a time; pour, read, record, return, repeat)
- Manual data processing steps (handheld calculators came very late)
- No software to process non-linear reactions; had to convert data to linear format and manually draw best fit line

Potency Bioassays for Biological Products: Then vs Now



NOW (1970's to present day)

- Well-characterized biological products (inc vaccines) and CGT products
- Few products still need in vivo potency bioassays; most have converted to in vitro bioassays for product release and stability testing (ethics)
- Most in vitro bioassays use microtiter plate format with expanded replication scheme (inc multiple plates)
- Microliter-scale reactions with calibrated variable-volume pipettors
- Disposable tips and microtiter plates (no washing artifacts)
- Better commercially-available critical reagents and plates; characterized cell lines and purified ligands
- Many more highly sensitive reaction detection modalities (chromogenic, fluorescent chemiluminescent, electrochemical)
- Better controlled equipment for incubation of bioassay plates (rare edge effects)
- Better analytical instrumentation for more sensitive, more accurate measurements (eg meniscus corrections)
- Computerized instrument operation (digital settings, not analog dials)
- Rapid data collection steps (all 96 wells simultaneously)
- Better data processing software; can handle complex non-linear reaction results

NOW: Bioassay Validation and Development Guidance

ICHQ2 (Q2A 1994 / Q2B 1996; merged into Q2(R1) 2005)

- Alluded to (but did not define) reportable vs working ranges (Q2A: “linearity is a subset of range”; Q2B: “range is a subset of linearity”)
- Defined and described the core parameters of method validation for physiochemical methods, but excluded biological assays
- But specificity, linearity, range, accuracy, intra-assay precision (repeatability), intermediate precision were fairly easily adapted to in vitro bioassays

USP <1033> (2013 initial; under revision now for 2026)

- Covers every type of potency bioassay (in vivo, ex vivo, in vitro) with one set of recommendations
- One-size-does-not fit all: animals, tissues, cell-based, ligand binding, lytic, and enzymatic bioassays
- Focuses only on validation experiments to cover the product potency reportable range (linearity, accuracy, precision); does not describe validation of the method’s working range
- Describes partial intermediate precision validation options (ie not the full procedure to reportable results)
- During method validation allows revision of method replication scheme ‘on the fly’ if needed to pass
- Utilizes highly complex statistical strategies even for well-defined and well-controlled in vitro bioassays (not wrong, but can obfuscate underlying method performance issues)

NOW: Bioassay Validation and Development Parameters

ICHQ2(R2) (2023)

- Specifically includes in vitro bioassays along with advanced physiochemical methods
- Still excludes in vivo / ex vivo bioassays
- Defines and describes reportable range vs working range; clarifies that both require validation
- Adds information on ‘relative accuracy’ for in vitro potency bioassays

ICHQ14 (2023)

- Provides a highly detailed example of systematic development of a cell-based potency bioassay (aspirational!)
- Describes critical assay parameters to assess in order to optimize method operational robustness
- Does not clarify that optimization of robustness is different from confirmation (validation) of final method robustness ranges

FDA Guidance Analytical Method Validation (2015)

- States that method development data can be used to support method robustness IF the historical data are
  - Relevant to the current version of the method SOP
  - Bracket the operational ranges allowed in the method procedural steps
  - All data are documented with auditable data integrity for future GMP inspections
  - All data are presented in detail in the method validation report (not just referenced)

NOW: Understanding Bioassay Reportable vs Working Range

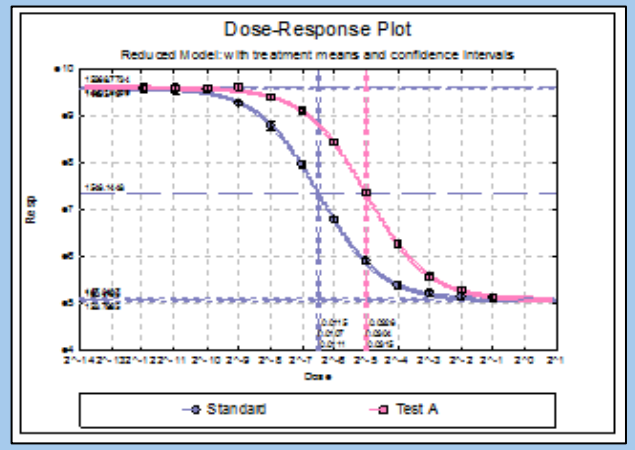
Method WORKING Range

- *Range of the bioassay response factor (eg Upper/Lower limit of DR curves, enzyme reaction Vo – Vmax)*
- *Performance characteristics are embedded in each run of the method; used in validated method SOPs as SST criteria*

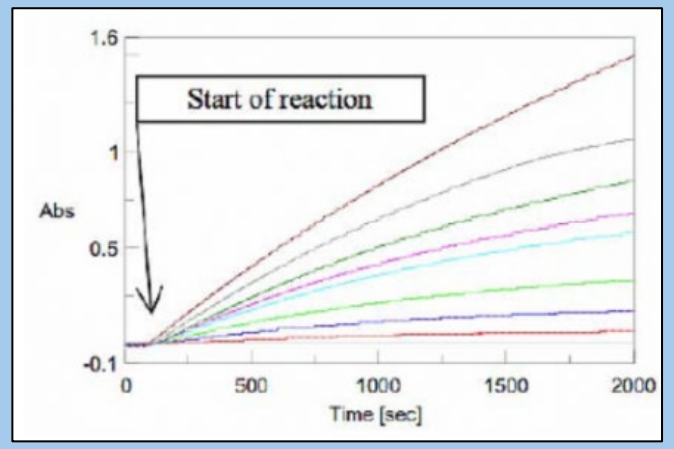
Test Sample REPORTABLE Range

- *Range of the potency specification to be tested with that bioassay (eg 75% - 125% Relative Potency)*
- *Performance characteristics are validated by independent intermediate precision runs with test samples that bracket the spec*

Binding Reaction **Working** Range

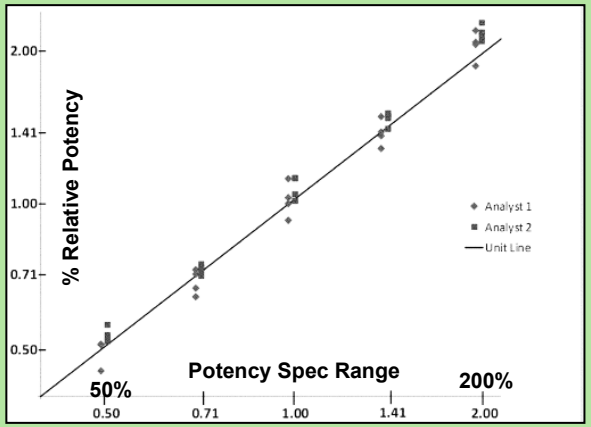


Enzyme Reaction **Working** Range



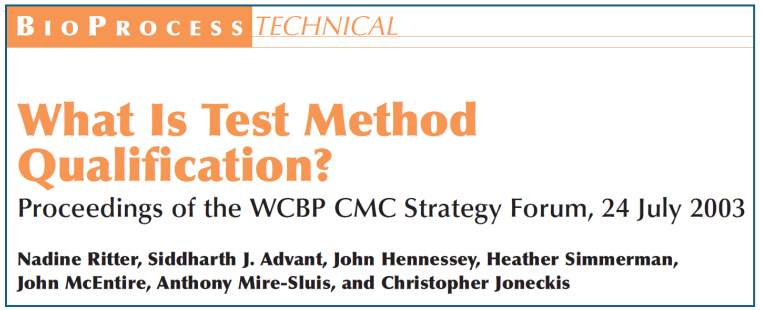
Data points are generated in each run of the method SOP

Product Potency **Reportable** Range



Data points are 30 runs of the method SOP

NOW: Bioassay Qualification vs Phase-Appropriate Validation


BIOPROCESS TECHNICAL

What Is Test Method Qualification?

Proceedings of the WCBP CMC Strategy Forum, 24 July 2003

Nadine Ritter, Siddharth J. Advant, John Hennessey, Heather Simmerman, John McEntire, Anthony Mire-Sluis, and Christopher Joneckis


Phase-Appropriate Method Validation Strategies: Policies and Practices to Support Clinical Development

WCBP CMC Strategy Forum, January 2018

Nadine Ritter, *Global Biotech Exerts, LLC, USA;* Al Del Grosso, *CBER, FDA;* Brian Janelsins, *CDER, FDA;* Sandra McSheffrey, *Health Canada, Canada*

Both 2003 and 2018:
QUALIFIED methods must be used for
✓Process development or process validation (not for routine cGMP manufacturing)
✓Product characterization and comparability (not for routine cGMP release or stability testing)

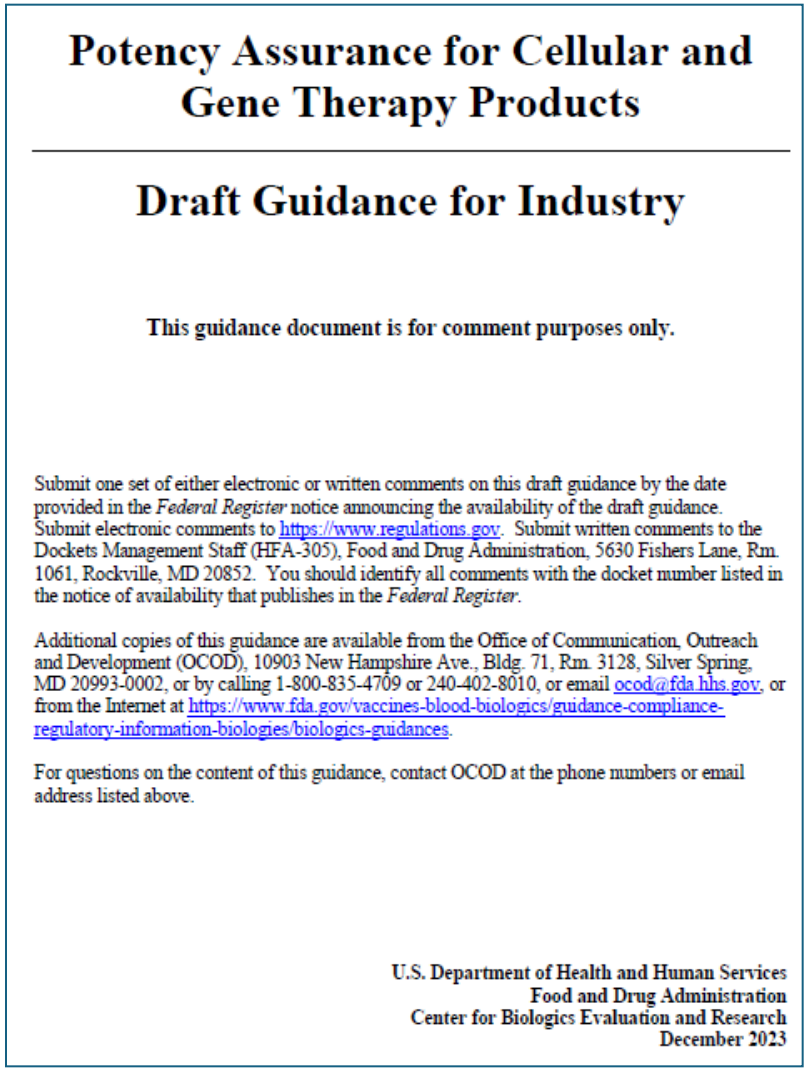
Added in 2018:
QUALIFIED methods must also be used for
✓Methods used for process Quality by Design and to confirm small scale model comparability to large scale process
✓Methods used for biosimilar product comparative analytical assessments (CAA)

Old 2003:
- QUALIFIED methods may be used for early phase QC test methods that will eventually be VALIDATED
- Late phase and commercial product QC test methods must be FULLY VALIDATED for GMP compliance

New 2018:
- QC methods for clinical trial material must be VALIDATED (GMP compliance term) not just QUALIFIED
- Method validation should be 'PHASE APPROPRIATE" in terms of rigor and robustness

BUT Still no official guidance documents answered the original 2003 CMC Forum question:
WHAT *IS* TEST METHOD QUALIFICATION?

NOW: Definition of Method Qualification vs Phase-Appropriate Validation



Potency Assurance for Cellular and Gene Therapy Products

Draft Guidance for Industry

This guidance document is for comment purposes only.

Submit one set of either electronic or written comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-8010, or email ocod@fda.hhs.gov, or from the Internet at https://www.fda.gov/vaccines-blood-biologics/guidance-compliance-regulatory-information-biologies/biologics-guidances.

For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
December 2023

3. Assay Qualification and Validation

Assay **qualification** involves **determining** the assay's performance characteristics (e.g., accuracy, precision, specificity, and sensitivity).

Qualifying a potency assay allows one to determine whether assay performance is adequate for the intended purpose of helping to assure product potency, or whether assay performance instead needs to be further optimized.

Potency assays should be qualified as soon as feasible, and no later than the initiation of clinical investigations that are intended to provide substantial evidence of safety and effectiveness for a marketing application.

DP release assays for a licensed product must be validated. Assay **validation** should **confirm** the performance characteristics of the fully-optimized assay by comparing assay performance during the validation study to appropriate pre-specified acceptance criteria for accuracy, precision, specificity, and other relevant performance characteristics.

“A Smooth Sea Does Not Make a Skillful Sailor”

Lessons Learned from Above and Below Decks About Bioassay Shipwrecks

https://fishofgold.net/2015/01/23/thar-be-dragons/

In Vitro Bioassays: Issues Encountered Above Decks (FDA)

1. Cell Based Assays

a) Long-term stability of assay cell bank (engineered cells)

b) Bank homogeneity / lack of contamination

c) Growth rate and cell viability in expansion steps

d) Cell passage number range (min/max for method working range)

e) Adherent vs suspended cells (critical pipetting technique)

f) Extracellular supernatant vs cell lysate (critical pipetting technique)

2. Ligand Binding, Immunologic, or Enzymatic Assays

a) Concentration and purity of each lot of commercial or custom antigen / antibody / enzyme / substrate

b) Lot to lot variability of commercial or custom detection reagent (signal differences)

c) Lab handling of assay reagents (multi-use contamination, temp cycling; pooling remainders)

3. All plate-based assays

3. Type of microtiter plate (composition of materials)
4. Type of plate cover (adhesive or loose)
5. Make / model of plate reader (default settings)
6. Type / version of plate reader software (data processing steps)
7. Shift in assay window with changes in critical assay materials / reagents

4. Ref Std, Assay Control, and Test Samples

3. Batch preps of Ref Std and Assay Control (F/T stability of aliquots)
4. Hold time / temp of Ref Std, Assay Control and Test Samples prior to testing
5. Differences in test sample matrices (IPC vs DS vs DP)

In Vitro Bioassays: Issues Encountered Above Decks (FDA)

1. Cell Based Assays

a) Long-term stability of assay cell bank (engineered cells)

b) Bank homogeneity / lack of contamination

c) Growth rate and cell viability in expansion steps

d) Cell passage number range (min/max for method working range)

e) Adherent vs suspended cells (critical pipetting technique)

f) Extracellular supernatant vs cell lysate (critical pipetting technique)

2. Ligand Binding, Immunologic, or Enzymatic Assays

a) Concentration and purity of each lot of commercial or custom antigen / antibody / enzyme / substrate

b) Lot to lot variability of commercial or custom detection reagent (signal differences)

c) Lab handling of assay reagents (multi-use contamination, temp cycling; pooling remainders)

3. All plate-based assays

3. Type of microtiter plate (composition of materials)
4. Type of plate cover (adhesive or loose)
5. Make / model of plate reader (default settings)
6. Type / version of plate reader software (data processing steps)
7. Shift in assay window with changes in critical assay materials / reagents

4. Ref Std, Assay Control, and Test Samples

3. Batch preps of Ref Std and Assay Control (F/T stability of aliquots)
4. Hold time / temp of Ref Std, Assay Control and Test Samples prior to testing
5. Differences in test sample matrices (IDS, DS, DP)

Model FDA Case Studies: Change in Type of assay Plates (PAS)

Potency Assay change: The Microtiter Plate

C. Fuchs - USP's 3rd Bioassay Workshop August 11-12, 2010

- Assay variability was too high
- Observed a time-dependent loss in activity due to non-specific binding to plate A.
- So, sponsor changed plate A to a "non-binding surface" plate B.
- Post-change assay was validated using only intermediate precision.

FDA told Applicant that the assay was not appropriately validated. Required full re-validation of the method and a head-to-head comparison between Plate A and Plate B

- ✓ New validation study included Accuracy, Precision, Range, Linearity, as well as a Head-to-Head comparison
- ✓ The validation exercise identified that the change in plate resulted in shift in assay accuracy and precision
- ✓ Applicant wanted to change the specification acceptance criteria due to this shift in assay performance

FDA found it inappropriate to widen the acceptance criterion based on an increase in assay variability and asked that Applicant improve the assay precision

FDA requested that Applicant "*provide data along with a statistical analysis identifying the bias that has been introduced into the assay by the changes that have been implemented. A sound and robust analysis may permit you to revise the range of acceptable results based on this change in assay accuracy.*"

- ➢ Applicant increased the number of plates to sufficiently improve the precision of the assay.

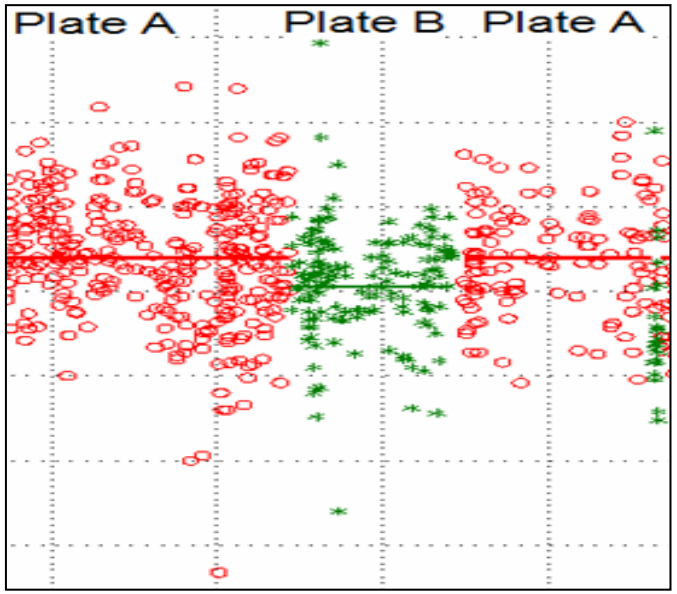
Model FDA Case Studies: Change in Type of assay plate (PAS)

Potency Assay change: The Microtiter Plate (cont'd)

Applicant evaluated the potency data for the Product Control Sample (PCS, single lot) through its history.

The dataset was for the PCS lot tested **>500 times using plate A** (red) and **>150 times using plate B** (green)

The tracking data showed introduction of plate B was associated with a decrease in the mean potency value of the PCS lot.



Potency acceptance criteria were revised by subtracting the total mean shift in potency from the upper and lower limits of the acceptance criteria to compensate for the change observed.

Approval of this plan was based on:

- Statistical analysis of the release and stability data for the protein
- The change to plate B where the values were lower than plate A.
- A separate *In vitro* activity study to support the new lower limit of the acceptance criterion.

Bioassay Issues Encountered when the Submission *Hits the FDA Deck*

1. **Inadequate Method Procedures**
   SOPs are not sufficiently detailed and robust
   a) For the potency assay procedural steps
   b) For controlling lots of critical reagents such as cells
   c) For implementing new lots of critical reagents
2. **Insufficient Assay transfers**
   a) SOPs must be detailed and robust
   b) Consider impact of site-specific differences (facility fit), e.g. Manual vs. automated liquid handling at different sites.
3. **Bioassay design to reflect the MoA**
   a) Assays are complex – need to design to be robust for QC function
   b) MoAs and products are complex – need to consider the science
   c) Design should include appropriate and sufficient controls
   d) Assays for product with multiple MoAs – product dependent and indication dependent.
   e) Not all bioassays will be used for lot release but they are needed for characterization, product stability impact, comparability, etc. e.g. bispecifics, ADCs, IFNa, TNFs, enzymes, CAA for biosimilars, etc.
4 **Inspection Observations**
   a) Issues with control and qualification of new lots of each critical reagent to maintain the validated assay ranges.
   b) Hidden data masking in routine runs was identified only inspection based on comment in the audit trail
   c) Duplicate cell supernatant samples were mixed before ELISA to reduce assay variability
   d) Data integrity issues for biosimilar development studies conducted outside of GMP lab

Bioassay Issues Encountered when the Submission *Hits the FDA Deck*

5. Inadequate Method Validation

a) Study not appropriate/sufficient for the assay used
b) Study not appropriate for all the formulations – e.g. DSI, DS, DP, different formats and formulation of DP.
c) Validation did not consider frequent change in critical reagents and materials
d) Validation of a stability bioassay was not validated to be stability indicating (no data using degraded samples)

In the BLA and on inspection it was apparent that Applicant did not plan for a lifecycle approach to ensure assay remains in the validated state

6. Changes in bioassays – insufficient data in method bridging comparability studies

a) For late development / BLA level change in bioassays, method bridging data should include:
   - Multiple lots of product
   - ICH stability samples (from real-time accelerated and target storage conditions)
   - Product stressed by various conditions, as relevant (e.g. temp, light, pH, oxidation, product variants, aggregates, samples from freeze/thaw studies, proteolytic degradants)

b) Full validation of the new/changed bioassay method

c) Studies correlating the old and new bioassays; Overlapping data using both assays are required to support assay change and link between assays, non-clinical and clinical data.

d) Include scientific understanding of any lack of correlation between the two bioassays with regards to stability indicating capabilities for the product.

Model FDA Case Studies: Bioassay Method SOP (PLI)

FDA reviews the potency assay SOPs as part of BLA review as well as on pre-license inspection (PLI).

- During BLA review FDA found the potency assay SOP significantly deficient in details, which triggered a series of IRs and telecons with Applicant, CMO and FDA to clarify the missing information
- The original SOP was translated into English for inclusion in the BLA and during the inspection
- In discussions we all seemed to be talking across each other, so FDA requested the SOP in the original language
- FDA found that the translation was at fault and the many issues FDA identified were not actually issues in the SOP that was used on site.
- However, this brought up an issue of insufficient control by the Applicant that used the same translated versions.

Well-written method SOPs are needed to minimize performance drift, prevent high bioassay variability, and maintain the bioassay parameters within validated state throughout the lifecycle of the product.

Model FDA Case Studies: Bioassay Method SOP (Late Phase IND)

Well-written method SOPs are needed to minimize performance drift, prevent high bioassay variability, and maintain the bioassay parameters within validated state throughout the lifecycle of the product.

- Bioassay was transferred to a CTO for the DP stability program (used for testing pullpoint samples)
- Original CMO lab continued to conduct DP release testing with same bioassay method
- Over time, the DP stability data at the CTO site showed increased degradation of the DP.
- Investigation initially covered DP manufacturing (eg. raw materials change etc), DP storage conditions, and eventually the potency assay SOP.
- Investigations identified that tweaks made by the CTO to some steps in the method SOP resulted in the changes to the potency assay results for the stability studies.
- The bioassay SOP was the same at both sites, but it was not detailed enough to prevent site-specific tweaks that had a tangible impact on method performance.

Model FDA Case Studies: Bioassay design for the MoA (BLA)

Multiple isoforms of the same target - are all equally relevant for use in the bioassay?

Example: VEGF (target of VEGF antagonists such as bevacizumabs, aflibercept, ziv-aflibercept etc.)

Targeting VEGF results in prevention of VEGF-mediated endothelial cell growth and proliferation, and angiogenesis. Bioassays measuring the inhibition of VEGF-mediated endothelial cell growth directly reflect the MOA of these molecules.

Are all VEGF-A isoforms equally relevant in the bioassay?

VEGF-A is present in several isoforms of which VEGF-A165 and VEGF-A121 are the most predominant physiologically. VEGF-A165 can be cell-associated through its heparin binding domain. Other VEGF-A isoforms are also identified in different tissues. ***Which of the many VEGF- A isoforms should be used in the potency assay for antibodies to the different indications? Are they equally physiologically relevant and equally functional in the bioassay?***

During the BLA review for an oncology indication Applicant was asked to support their use of a specific VEGF-A isoform that is not usually seen in tumor cells but was used in their bioassay.

Subsequent studies included comparison of binding kinetics of the protein to that isoform as compared to other isoforms predominantly seen in the targeted oncology indications and more predominant and physiologically relevant.

Model FDA Case Studies: Bioassay Design for the MoA (PLI)

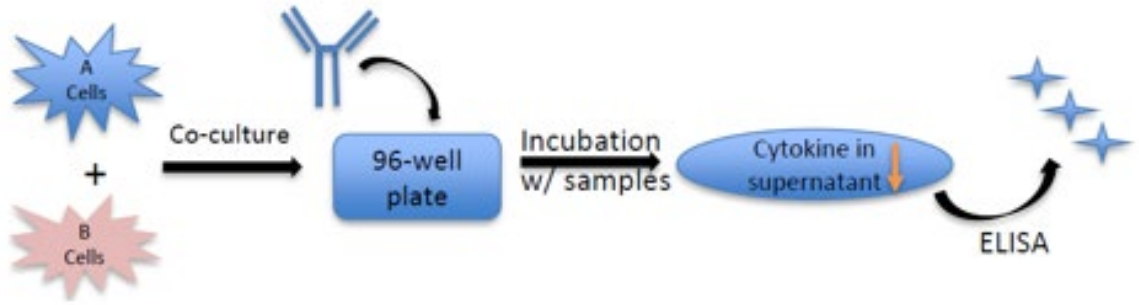
Leiyun Boone Bioassays, April 16, 2024

Product and MoA

- Product is humanized antagonist IgG1-Mab
- Fc is engineered to silence potential effector functions
- Binds to membrane target
- Induces immune activation for oncology indication

Bioassay

- Uses co-culture of two types of cells:
  - Cell A is engineered to express the target
  - Cell B is engineered to express target receptor
  - Co-cultured cells lead to cytokine expression
- Assay measures the Mab's ability to block cytokine expression, measured by ELISA
- Samples are tested in duplicate; SST requires CV of duplicates ≤ 30%

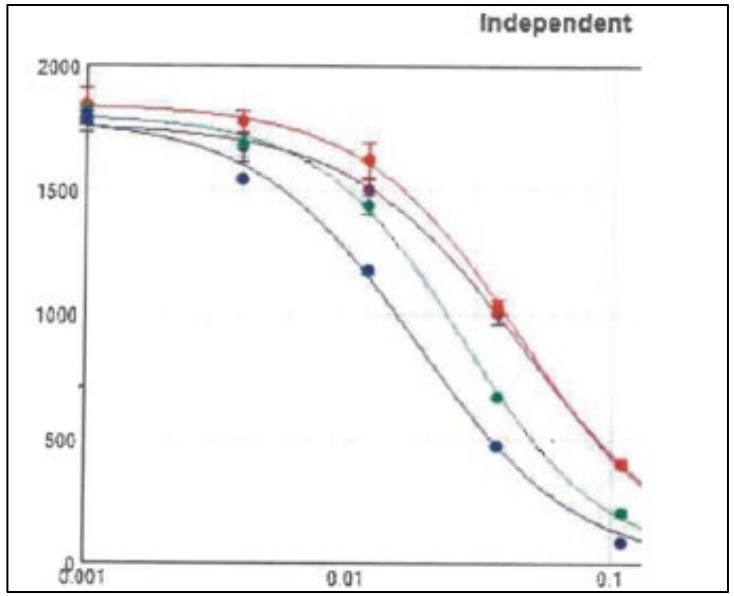


Model FDA Case Studies: Bioassay Design for the MoA (PLI)

Leiyun Boone Bioassays, April 16, 2024

During Inspection it was found that:

- Invalid runs were due to high %CV
- It was found that the cell culture supernatant from the duplicate sample preps were mixed prior to the ELISA step to “reduce variability in replicate % CV”
- Inadequate SST and curve similarity controls (ie controls for hill slope but not upper or lower asymptotes
- High variability at the upper asymptote



Assay format was very complex and NOT QC Friendly

Assay lacked appropriate SST criteria for procedural control in each run of the method

Model FDA Case Studies: Bioassays for Multiple MoAs (IND)

Bispecific mAb: Sponsor claimed MoA had synergistic effect of the two binding targets

- The initial IND potency assays were designed to measure each target Individually by ELISA.
- FDA asked for a cell-based potency assay that better addressed the claimed synergistic MoA
- In response, sponsor deleted 'synergistic' from the MoA claim in the IND

With FDA's insistence, Sponsor ultimately developed an acceptable cell-based synergistic bioassay by late phase development

….and Yes, the bispecific mAb did have a synergistic effect of the two binding targets.

(Sometimes sponsors need a little FDA 'push' to meet the statutory requirement for potency assay)

FDA Expectation is that companies develop bioassays to support all claimed clinical MoAs (though some bioassays may be just for characterization / comparability studies)

- Biotechnology products can have more than one activity e.g. IFNa (anti-viral vs anti-proliferative)
- The product's structural / function requirements for different activities may differ
- A particular biologic activity may not reflect the structural requirements of active product
- Required structure/function relationships may be indication-specific.

If there are multiple possible mechanisms, more than one potency assay may be required, but that is a scientifically based decision looking at the holistic analytical package and at the science of the molecule

Model FDA Case Studies: Assay Change – Method Comparability (PAS)

Potency assay method bridging comparison studies should include side by side testing of:

- Multiple lots, including different strengths and formulations, as relevant.
- Real time ICH stability samples (Accelerated and Target temperature conditions)
- Stressed samples (e.g. photo-exposed, UV induced dimerization, enriched aggregates, proteolyzed samples including low and high % spikes (some deg conditions may be product specific))

	Bioassay #1		Bioassay #2	
Samples and Conditions	Mean	%CV	Mean	%CV
Control, Normal Storage Temperature	96	8	99	5
Elevated Temperature – short term	86	8	71	4
Light Protection	99	6	97	2
High Light Exposure	70	5	47	3
0% Oxidizing Agent	103	8	100	4
5% Oxidizing Agent	94	7	80	4
Acidic pH Conditions	97	4	86	1
Basic pH Conditions	90	5	77	3

Comparison of stability-indicating capabilities was assessed using stressed product samples from various physical and chemical conditions

Bioassay #2 is equivalent or better for the stability indicating parameters and has slightly better precision.

In Vitro Bioassays: Issues Encountered Below Decks

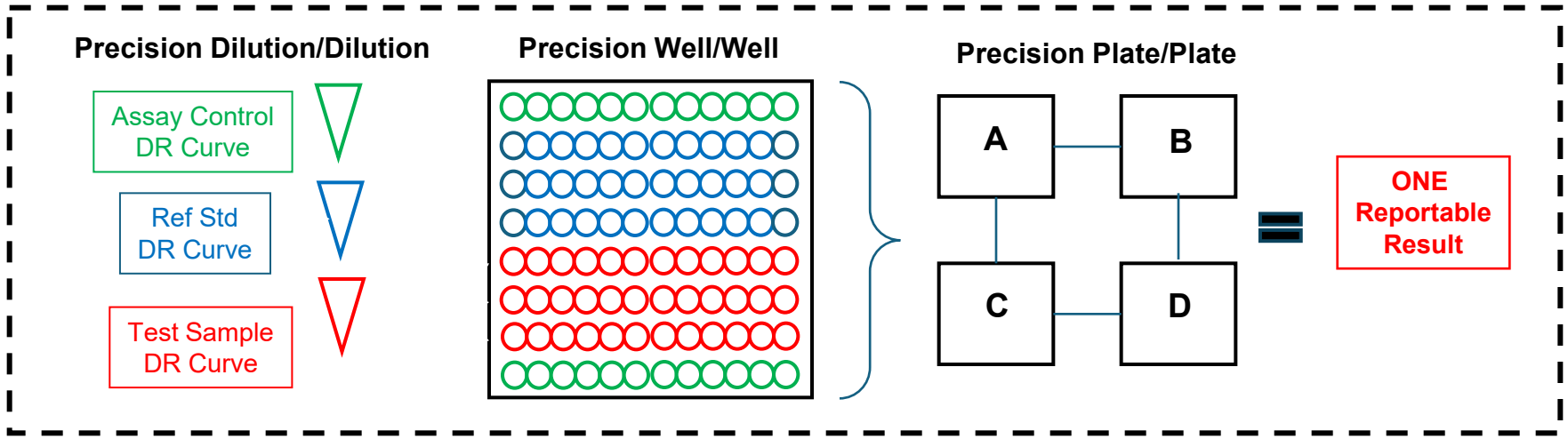
1. **Detection range of dose response curve**
   a) OD range between upper/lower asymptotes of Ref Std (assay window)
   b) Shift in assay window with Test Samples (hockey-stick effect)
2. **Number of points in dose response curves**
   a) 8 (vertical columns)
   b) 10, 11, 12 (horizontal rows)
3. **Replication scheme**
   a) Duplicate vs triplicate wells for each curve point
   b) True replicate or pseudo replicate dilutions
   c) Number of plates required to generate reportable result
   d) Multiple plates: Replicates placed within each plate or between plates
4. **Plate layout**
   a) Adjacent vs alternating rows/columns (check plate bias)
   b) Fully randomized wells (check analyst error rate)
5. **Volumes / concentrations at each step**
   5. High vs low concentration solutions (pipette carryover)
   6. Large volume dilutions (pipettors above 1000 ul)
   7. Small volume transfers (pipettors below 20 ul)
   8. Manual vs automated liquid handling (fluid channel carryover)
   9. Single vs multichannel pipettors (state of calibration at time of use)
6. **Incubation / detection steps**
   5. Total volume in each well (surface loss)
   6. Covered or uncovered wells (edge effects)
   7. Plate homogeneity (temperature, light, shaking)
   8. Controlled or ambient environment (variations in lab sunlight, HVAC)

Below Decks: Intra-Assay Precision vs Inter-Assay Precision

"Intra-assay" (Precision, repeatability) means all of the steps required to generate ONE REPORTABLE RESULT from the method procedure

"Inter-assay" (Intermediate precision) means generating INDIVIDUAL REPORTABLE RESULTS by running the method procedure on different days, with different analysts, using different instruments

INTRA ASSAY PRECISION (REPEATABILITY)



INTER ASSAY (INTERMEDIATE) PRECISION

Run 1 Reportable Result	Run 2 Reportable Result	Run 3 Reportable Result
Run 4 Reportable Result	Run 5 Reportable Result	Run 6 Reportable Result

Intermediate Precision Should Compare:
- Day to Day
- Analyst to Analyst
- Instrument to Instrument

Overall Intermediate Precision:
- Pool of Days, Analysts, Instruments

Below Decks: Badly Written or Poorly Controlled Bioassay Method SOP

Bioassay SOPs should be written SPECIFICALLY and UNAMBIGUOUSLY to support reliable, consistent conduct of the procedure in the laboratory by any appropriately trained analyst each time it is run.

Make Bioassay Procedural Steps Tight Until / Unless Data Support Flexibility

- *Early phase method SOPs should only allow options and ranges with existing data*
- *Increase SOP options and widen ranges only with relevant operational robustness data*
- *Control variations in critical materials and reagents by requiring method performance qualification*

Assure HISTORICAL POTENCY DATA CONTINUITY

by forcing METHOD OPERATIONAL CONSISTENCY

SOP can have all of the flexibility for which there is relevant robustness data

Below Decks: Inadequate Bioassay Method SOP Instructions

“Dilute in MilliQ water”

Is water actually suitable for the sample types?

“Dilute 1:1” (or 1:2?)

Give specific examples to avoid confusion, “eg 50 ul A plus 50 ul B”

“Suggested Dose Response curve points”

What is the method’s validated dose response points? I suggest you use that…

“Trypsinize the cells”

For how long? At what temp? with what amount of enzyme?

“Incubate until color develops”

What is the optimized reaction time to assure consistent signals? And why would it change from run to run?

“Graph the Dose Response Curve”

How to process the raw data points: all individual values, or mean of replicates?

“Use a suitable curve fitting program”

What defines “suitable” for this type of dose response curve, and why would it change from run to run?

Below Decks: Prep/Handling of Test Samples, Standards, and Controls

Specifically indicate in each **bioassay method SOP** how release and stability test samples are to be:

- Stored upon receipt in the QC lab (temp; critical for stability pullpoints)
- Thawed or Reconstituted at first use
- Stored after thawing/reconstitution (temp, time)
- Processed for each test (method specific steps)
- Held at the bench pending analysis (eg in before going into plate reader)

Specifically indicate in each **bioassay method SOP** how reference standard and assay control are to be:

- Thawed or Reconstituted at first use
- Prepared for routine QC use (batched prep with aliquots, or fresh preps each run?)
- Single use aliquots, or maximum number of F/T cycles allowed

Specifically indicate in a **protocol and report** **how bioassay reference standard and assay control** are to be:

- Calibrated for response factor (Ref Std) or relative potency (Assay Control) in the method
- Assessed for F/T stability (if stored as frozen aliquots)
- Monitored for real-time stability (if stored as frozen aliquots)

Below Decks: Clearly Define the Raw Data Processing Steps in SOP

Specifically indicate in each test method SOP even / especially if these steps are programmed into the bioassay software application:

- How many replicate samples will be analyzed
  - *Do not jeopardize accuracy for high throughput – MORE REPLICATES, FEWER SAMPLES*

- How many replicates from each sample (pseudo-replicates) will be run in the plate(s)
  - *Three really is a magic # for precision SST – ALLOWS OUTLIER MASKING TO SAVE POINT*

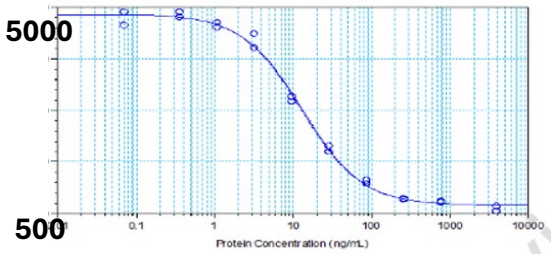
- How many plates are required to execute one complete run of the method
  - *One plate for the final reportable result is highly risky – THREE-PLATE STRATEGY WORKS WELL*

- How the individual data points will be processed to generate the final reportable result that will be compared to the product QC acceptance criteria
  - *Each plate is an intermediate result, not reportable result – FDA OOS GUIDANCE*

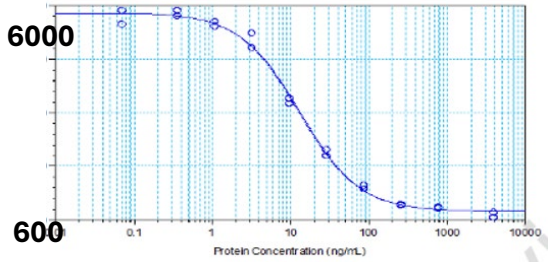
Complex statistical plate format: Not wrong, but just because you CAN does not mean you SHOULD

Below Decks: Raw Data Reveal Reality Behind Relativity

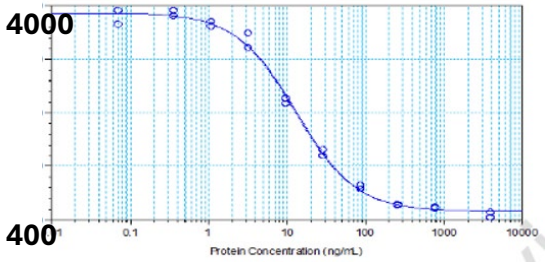
Established Working Range



Normal Variability Up

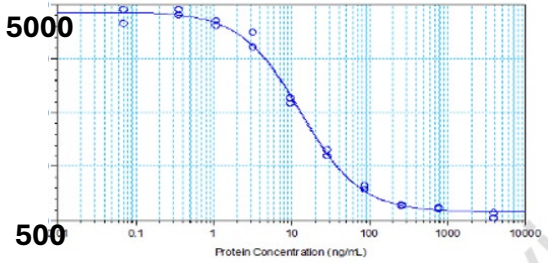


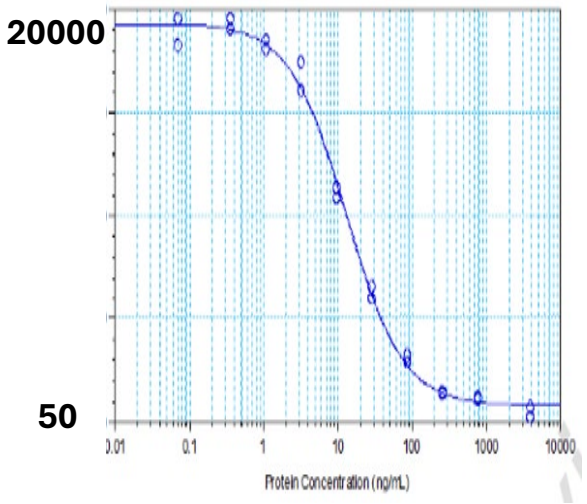
Normal Variability Down

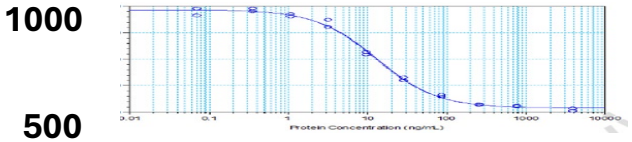


Constant A/D Ratio

Established Working Range







Variable A/D Ratios

Drifting A/D Ratios

Experiences Below Decks: MCB/WCB for Cell Based Assays

Practical considerations for the characterization and control of MCB/WCB for cell-based bioassays

Experience is What Let's You Recognize Mistakes the SECOND Time

INFORMATION REQUIREMENTS FOR BIOASSAY TROUBLESHOOTING ASSIGNMENTS

1. Complete bioassay method SOP(s) including instrumentation and software application for the exact method workflow (start to stop) and procedures for qualification of new critical reagents

2. Complete bioassay method validation protocol and validation report, including all raw and processed data from each validation run, and the tally of valid vs 'invalid' runs that occurred during validation

3. Copies of complete data sets (raw ODs to 4PF and % potency calculations) from examples of good bioassay runs and bad bioassay runs, with annotated outlier/masked wells (if present)

4. Chronological test date mapping of bioassay runs conducted for stability pullpoints (especially if samples from multiple stability studies are pulled and tested in groups)

5. Tracking/trending data on the frequency and nature of bioassay invalid runs during routine use after initial validation

MOST OF THE TIME THE ISSUES WERE THERE ALL ALONG – BUT NOBODY NOTICED!

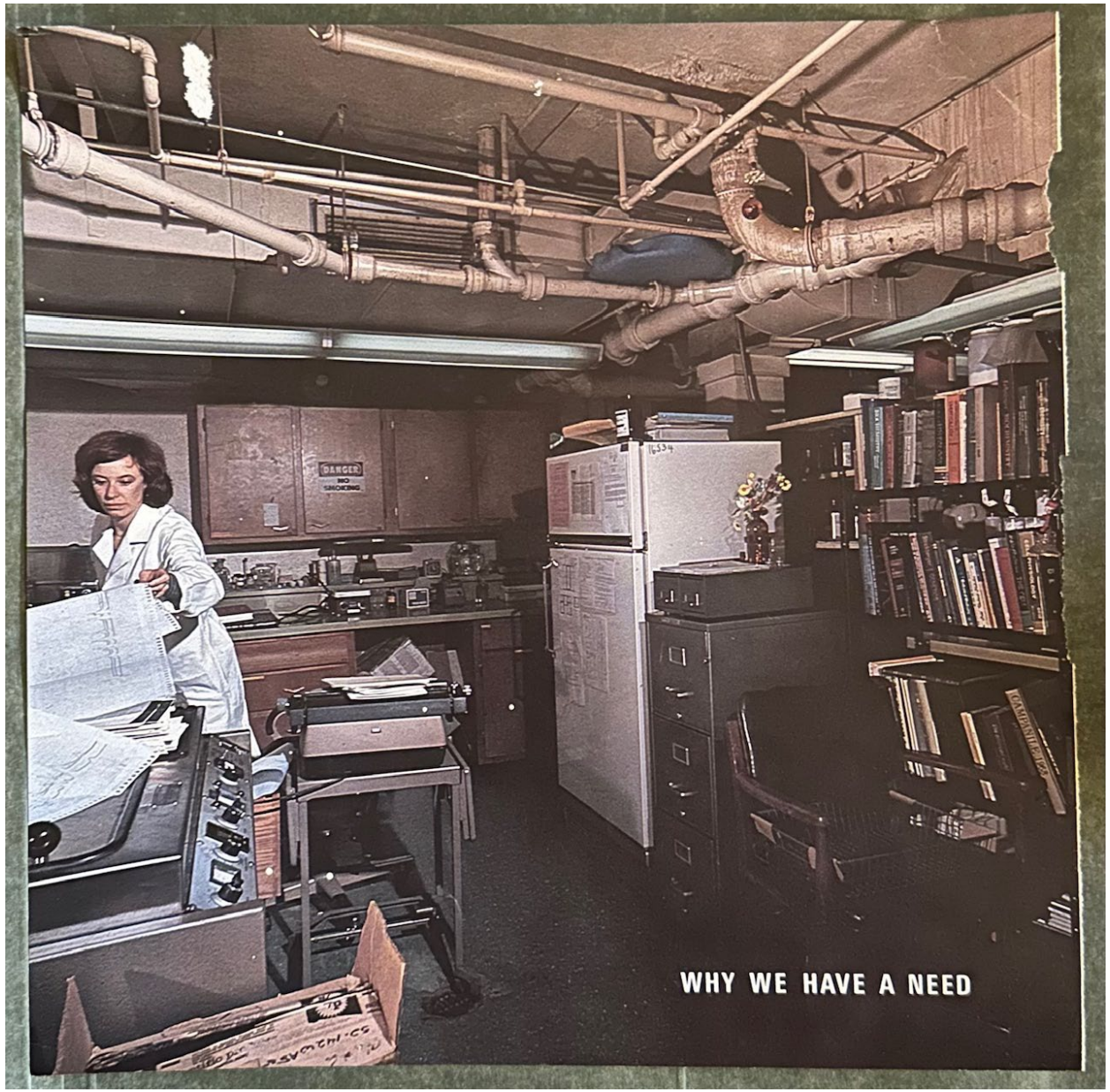
MY ACTUAL FIRST JOB IN AN ANALYTICAL LAB

1978 UT-HSC HOUSTON

ALL EQUIPMENT WAS DONATED FROM 1940'S OIL COMPANY LABS AND 1960'S NASA LABS

THIS PHOTO WAS TO BEG FOR GRANTS TO UPDATE THE LAB!

(LARGE PIPES WERE THE UPSTAIRS TOILETS)



Notable Historical Female Scientists *(Besides Us, Of Course!)*

Emilie du Chatelet
1706 – 1749

THANK YOU!

Mary Somerville
1780 - 1872

At age 27, she began studying mathematics seriously and then branched into physics. Historical evidence indicates that Du Chatelet's work had a very significant influence on the philosophical and scientific conversations of the 1730s and 1740s. Her works were published in the highest scholarly journals of the time; she was famous and respected by the greatest thinkers of her time. Her most recognized achievement is her philosophical magnum opus, *Institutions de Physique* (*Foundations of Physics*).

At age 14, she delved into the study of algebra and mathematics, defying her father's injunction against such pursuits. Somerville conducted experiments on the blackening effect of sunlight, the reaction used in early experiments in photography. She also produced an early version of the optical spectrometer. Throughout her life, she published series of original writings on astronomy, chemistry, physics and mathematics that were acknowledged around the world.

https://www.smithsonianmag.com/science-nature/ten-historic-female-scientists-you-should-know-84028788/