
# Welcome to BEBPA's

*Sneak Peek Webinar*

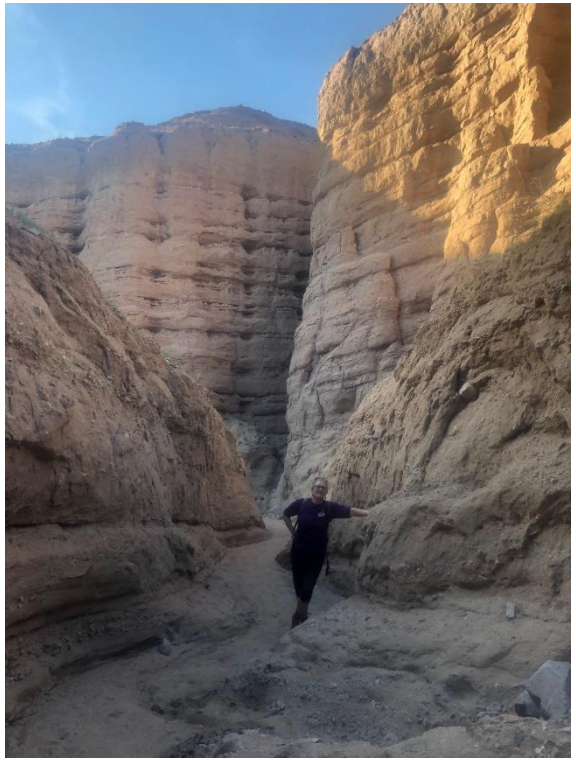
## Bioassay Basics Workshop

Begins at :

Korean Time 9:00 AM

USA 5:00 PM (PT)

# Welcome & Introduction



## Introduction

- Experience: Working in industrial biotechnology since 1986. Consulting for small and large biopharmaceutical firms since 1993. Working on Analytical Test Procedures for Commercial Release, CMC issues, Establishing GLP facilities and the founder of BEBPA which produces high quality conferences for the biopharmaceutical industry. I also founded and published BioQuality, a newsletter which monitored the regulatory and compliance happenings in the industry.

# Who is BEBPA?

Welcome to the Biopharmaceutical Emerging Best Practices Association (BEBPA), a non-profit organization dedicated to advancing patient safety through the collaborative efforts of the biopharmaceutical scientific community.

- 3+ Annual Hybrid Events
- 4+ Annual Virtual Events
- White Papers
- Free Scientific Webinars
- Technical Blog
- Journal Club
- Scientific Interest Groups
- Student Scholarships

# BEBPA Social Media







# Legal Stuff

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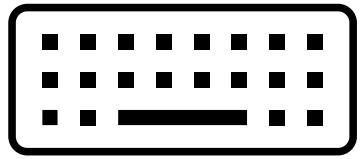
"Launch" by Hejum Ba (Whistle)

# Logistics

Enlarge the window to full size

Your Microphone is muted

Ask Questions in the Chat Box



You can always email questions later

# BioAssay Regulations During Development

**Good documentation: If you are working off of protocols be *very careful* to note the version # you are using.**

**All Equipment must be calibrated.**

**All reagents must be dated.**

**For bioassays – especially track your cells:**

- **Passage number, seeding ratio, handling procedures, etc.**

**If you are releasing clinical material: the best quality paradigm is cGMP – make sure that the quality unit releases all material before it is shipped.**

Crows at Sunset by Zeshin

## Practical Recommendation

*Note we will have a how-to session on this topic within the workshop at BEBPA APAC BioAssay Conference November 4-6, 2026 Incheon, South Korea*

If you are developing a ***cell-based assay*** pay attention to the cells. Focus your development effort on getting stable cells which are evenly distributed across your plate. (many labs are now using "thaw and go" or "ready-to-use" cells.

If you are developing an ***in-vivo assay*** pay attention to your injection procedure. If you are getting above 1-2% non-responders in your high dose animals, you probably have human error. Train with dye studies in recycled animals.

# What types of assays?

**ICH Q6B :**

“ Examples of procedures used to measure biological activity ....Animal-based biological assays ….Cell culture-based …. Biochemical assays …. enzymatic reaction rates or biological responses induced by immunological interactions”

“ Other procedures, such as ligand and receptor binding assays, ***may*** be acceptable ”

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# Guidances Specific for Bioassays

**Pharmacoepia:**

- EP 5.3
- USP 111 which is now 4 chapters 3 of which are 4-digit chapters: <1032>, <1033> <1034> and <111>

**FDA**

- Potency Assurance for Cellular and Gene Therapy Products released December 2023

**China**

- Chinese Pharmacopoeia 9401 Guidelines for Validation of Bioactivity/Potency Testing Methods for Biological Products

**Japan**

- Ensuring the Quality and Safety of Gene Therapy Products: PSEHB/MDED Notification No.0709-2 July 9, 2019

# Quality Risk Management and Assurance of Potency

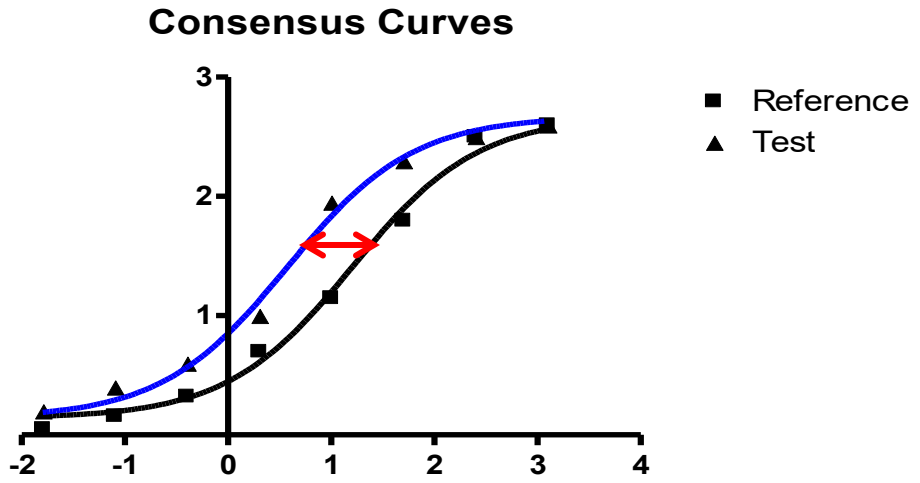
**Quality Target Product Profile (QTPP)** : Should include a summary of the potency-related characteristics of the product. The QTPP should be developed based on your understanding of the product's mechanism of action (MOA), the intended clinical indication and the route of manufacturing.

**Mechanism Of Action (MoA):** An understanding a product's MOA is important when identifying the product's potency-related Critical Quality Attributes (CQA). If you do not fully understand your product's MOA, ***you should continue to seek such understanding during development…products often have multiple activities and the specific activities that are most relevant to the therapeutic effect may depend on the targeted disease or condition***.

# Potency Assays are Relative

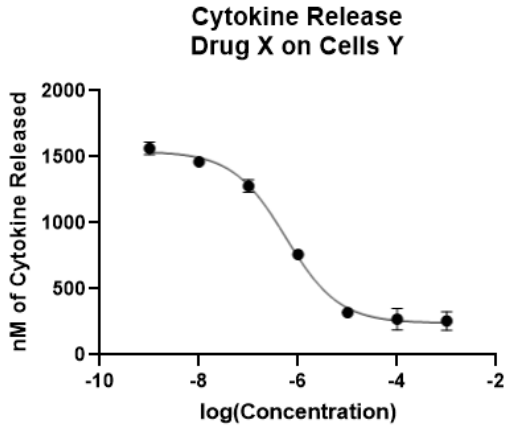
This means we run two full dose-response curves: The reference and the test sample.

Then we look at the shift of the dose response curve to quantify the potency.



# Step 1: A Dose-Response Curve

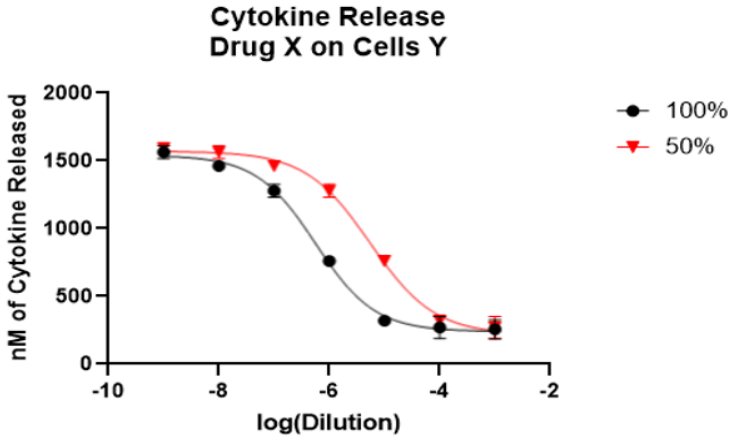
When we start developing a potency assay we concentrate on finding the right biology to link to the mechanism of action (MoA) and develop an assay to measure this read-out.


This is usually done with our batch of development material we were given by the manufacturing folks

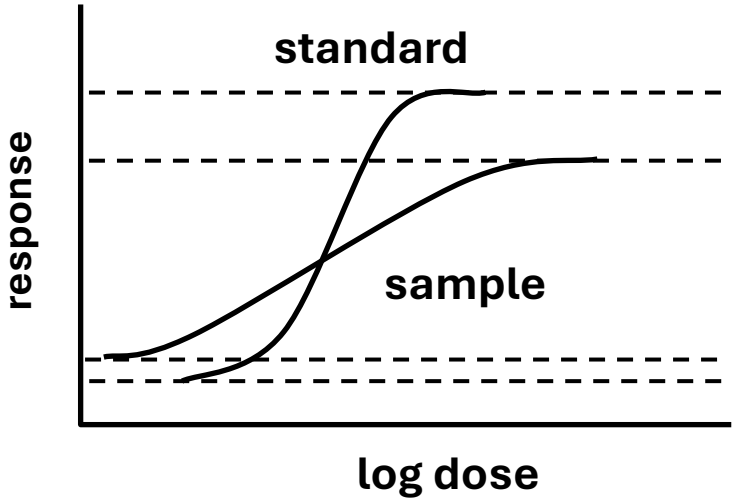
# Step 2: Let's see if 50% drug gives us a shifted curve!

- What do we do in this experiment?
- We make up two vials of drug
  - 100% concentration
  - 50% Concentration
- **<u>Run as if both were 100%</u>** (normal dilution scheme)

# Why Do We Do This? Scientific Answer

- Because our Read-Out System is not stable.
  - Therefore, we can't do an OD = a specific amount of protein.
  - Also, some products with inherent heterogeneity interact differently with different biological reagents.



# When Do We Need the Bioassay?

This is a question everyone asks – and there is no official guidance. However…..

**Phase I** Typically, you must show your plans for your product development. Have a binding assay if you have an antibody product. Always emphasize Talk the MoA and how you are developing a method to reflect this.

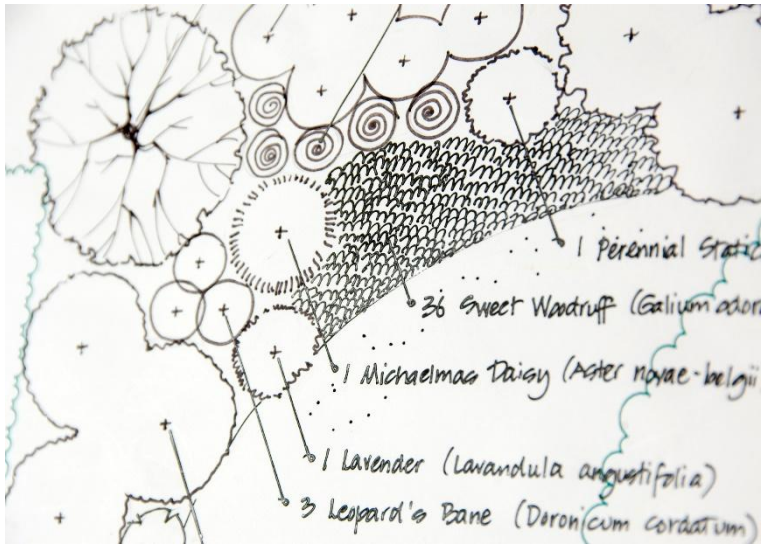
**Phase 2** – you should have a prototype potency assay (in a relative assay format) which will be used for releasing clinical material.

Why? Phase 2 is when you will be doing dose escalation, and we need to know the activity level being dosed so it can be compared to proposed dosing schemes in Phase 3 efficacy studies.

Companies will sometimes go on clinical hold if they do not have a potency assay by the start of Phase 2.

# State of the Phase 1 Assay

- Sometimes/Often it is not in a relative assay format
- Rarely is a specification attached to it.  Or if there is a specification it is quite broad.
- Often it is a simple binding assay or other characterization assay….*however*….
- You should have an understanding of the MoA and plans to have a cell-based (if appropriate) assay to share with the regulators



# State of the Early Phase 2 Assay

Most often are in the relative potency assay format

Interim product reference material – sometimes non-GMP material used in GLP

Qualified not validated

Usually use the “old” form of similarity assessment (We will talk about this in upcoming sessions)

# Case Study: Firms Put on Clinical Hold at PII

**Potency assay not qualified and ready to release clinical batches**

- Up until 10 years ago, this only happened between PII and PIII.
- Now it is more commonly seen between PI and PII.
- Often the regulators have been requesting to see the potency assay – but have not been provided with the data or a summary of analytical characteristics.
- Therefore, the formal request includes "validation". Usually, at this stage, a rigorous method characterization/qualification is all that is required.

**Result: Clinical hold not lifted until the method is "validated".**

# Nearing the finish line
## Early and Late Phase Three

***Use GMP reference material which is formally bridged to early reference material!!!***

Don’t Stop Working Now!

- Robustness studies should be completed prior to formal validation
- Formal assay protocol
- Well-characterized rare reagents (especially cells)
- Validation plan
- Ready to set commercial specification
- Switch to Equivalency approach to similarity if not done during Phase 2

# How do we choose which assay?

*Note: In the workshop at the APAC Bioassay Conference, we cover particular potency assays for a variety of different products*

Is the assay specified in pharmacopeia? May be tightly specified, or leave choice of possibilities (not usually an option for GT or Cell therapy products)

Scientific issues – does the assay assess the relevant characteristics of the molecule? (Mechanism of Action, starting now to discuss the matrix of assays – especially for complex products)

Logistical issues – do you have the appropriate infrastructure, equipment, expertise, resources?

Is it likely to meet performance requirements? (Watch out if you have required sample preparation)

Regulatory issues – is it likely to be acceptable to regulatory authorities?





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# One of the Most Critical Characteristics

**IS THE ASSAY STABILITY INDICATING?**

Ask this question early and often.  Work closely with your physical/chemical colleagues.

Obtain degraded, subpotent and aggregated material – ask how these products behave in your assay.

# Criticality of Linkage to MoA

"It is desirable that the assay(s) bear the ***closest possible relationship*** to the putative physiologic/pharmacologic activity of the product and be sufficiently sensitive to detect differences of potential clinical importance in the function of the product."

("PTC in the Manufacturing and Testing of mAb Products for Human Use" Feb 28, 1997)

# Selecting a Potency Assay

*Even if the clinical mechanism of action is known*,

- **Develop several activity assays –** These will ***all*** be run during development – down-selection to a single release assay (if possible) will occur just prior to becoming a commercial product.
  - Animal Assays (Vaccines and Toxins and some GT products)
  - Cell based Assays
  - Binding Assays (Antibody products and non-antibody proteins)
- Select the best behaved, most QC-proof assay ***which covers the MoA***, to be the potency assay. (But the ultimate choice will be up to the regulators)
- Use the rest for comparability studies and qualifying reference material.



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# What is the Matrix Approach to Potency Assays?

- It has different meaning for different product types.
- Gene Therapy – most sponsors take this to mean looking at the steps of getting a viral vector into a cell, looking for the expressed protein, looking at biological activity of the protein.
- Cell therapies – usually means the up/down regulation of multiple metabolic pathways within the cell product
- rDNA products – often means the ability of the therapy to bind a specific receptor and the triggering of a second messenger within the target cell.

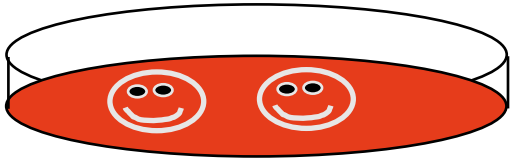
# Desired Correlation

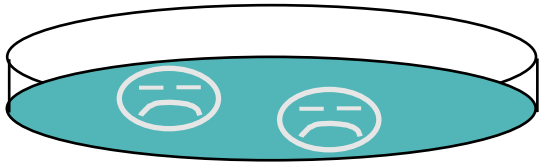
Almost Never Happens

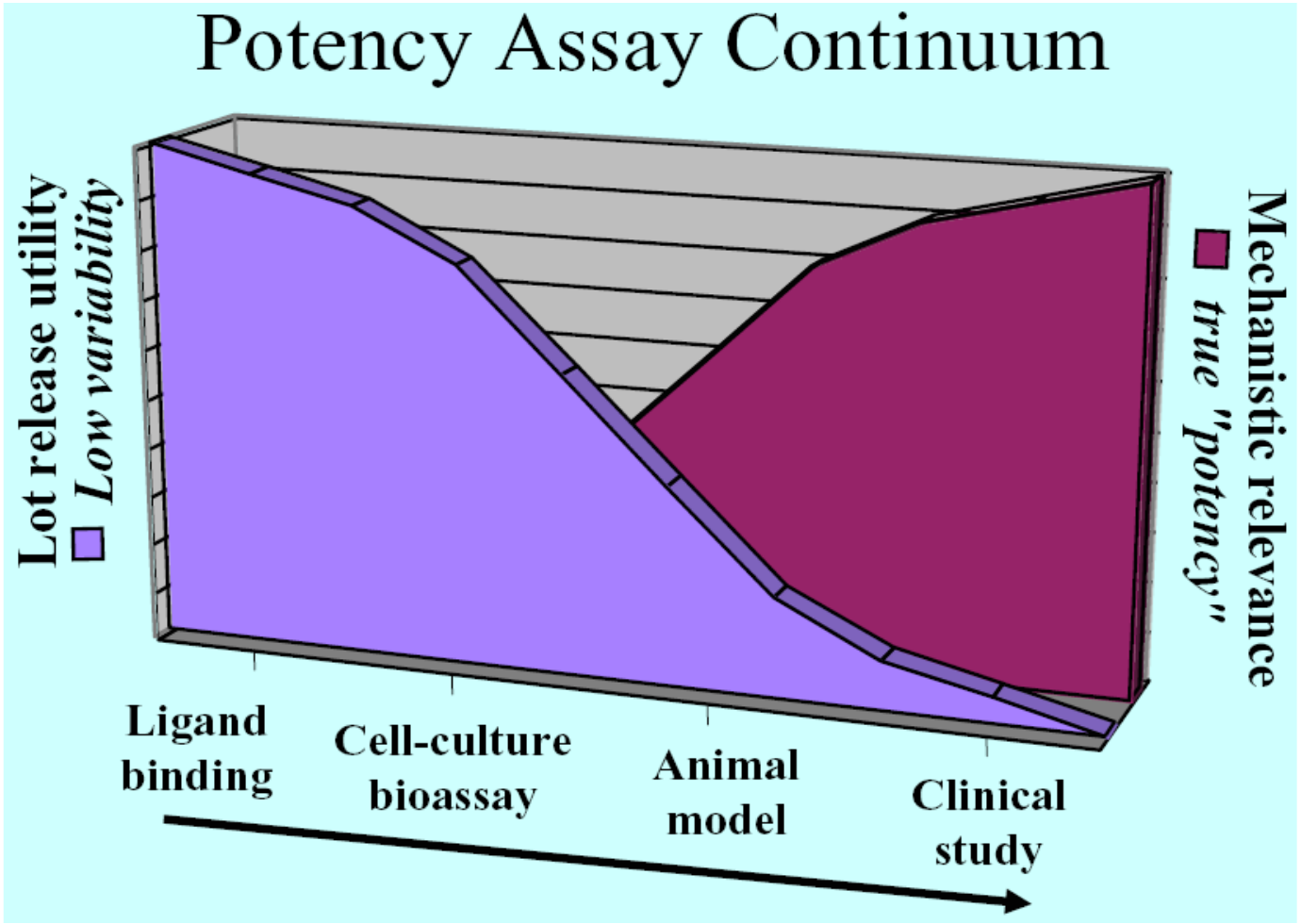
**between the expected clinical response & the activity in the biological assay should be established …. (ICH Q6B)**

**clinical performance**

**bioassay**







# Drug Substance vs Drug Product

- Depending on your product you may have different test strategies
- MoAb products often only do physical/chemical + binding on the DS as in process assays
- Then only do the cell-based assay on the DP for final release
- Why?  It is a risk assessment.  With a well-characterized product, it is rare for a product which has appropriate binding to fail the cell-based assay

# Any Last Questions Questions?

Free Webinar

# Design of Experiments (DoE) Basics for Analytical Assays

August 21st, 2026 @ 9:00 AM KST

Sign up here: https://bebpa.org/2026-aba/

# Thank You!

## Asia Bioassay Conference

November 4-6, 2026 | Incheon, South Korea

**$200 OFF Registration!**

Discount Code: ABAWEBINAR

You can register at https://bebpa.org/2026-aba/