# Reportable Values for Potency Assay

BEBPA 2017 Bioassay Survey

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President, BEBPA

# Before we start:

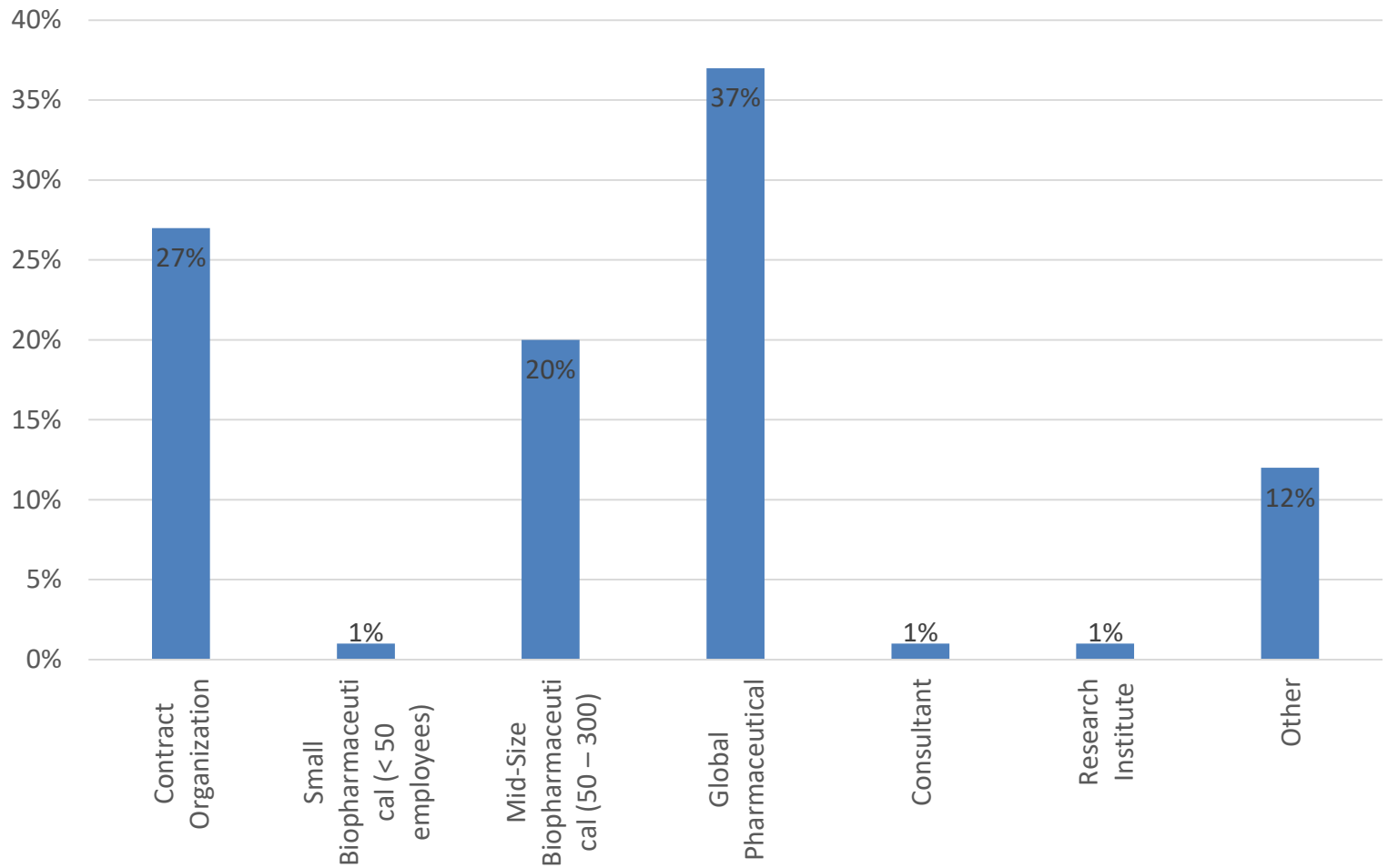
- You have a transceiver. These are to allow us to do some interactive things.
- When the clock appears in the bottom right hand side push a number for your answer.
- A green light will appear. If it remains green and then goes out your answer was accepted. If the light becomes red your answer was not received. Try again.
- If you hit the wrong answer – just answer again. The first answer will be removed and replaced with the most recent answer. (only 1 answer allowed per transceiver.)

# About You

## What Kind of Company do you work for?

1. Contract Organization
2. Small Biopharmaceutical (< 50 employees)
3. Mid-Size Biopharmaceutical (50 – 300)
4. Global Pharmaceutical
5. Consultant
6. Research Institute
7. Other

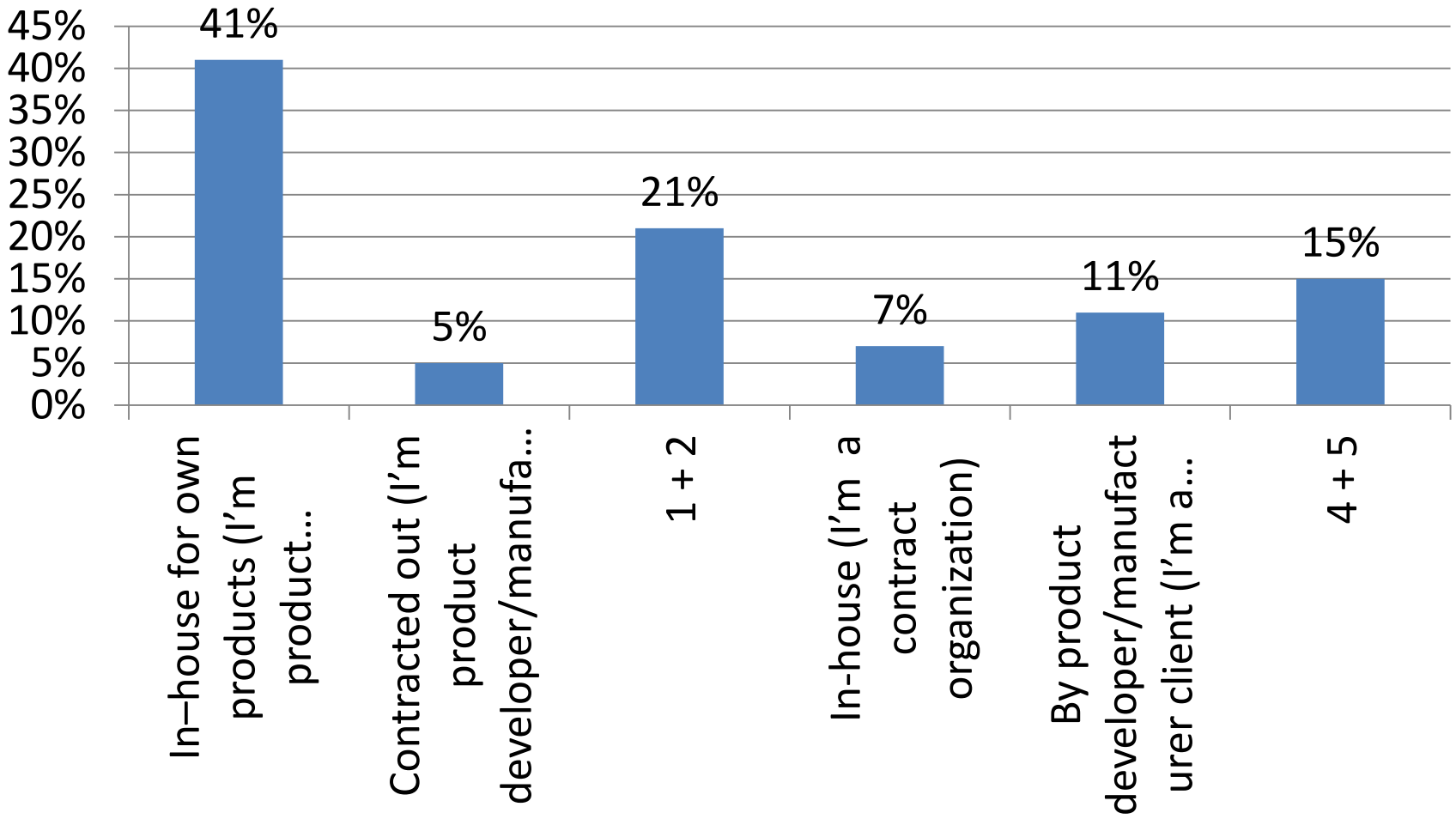
# What Kind of Company do you work for?



# Where are the bioassays developed?

1. In-house for own products (I'm product developer/manufacturer)
2. Contracted out (I'm product developer/manufacturer)
3. 1 + 2
4. In-house (I'm  a contract organization)
5. By product developer/manufacturer client (I'm a contract organization)
6. 4 + 5

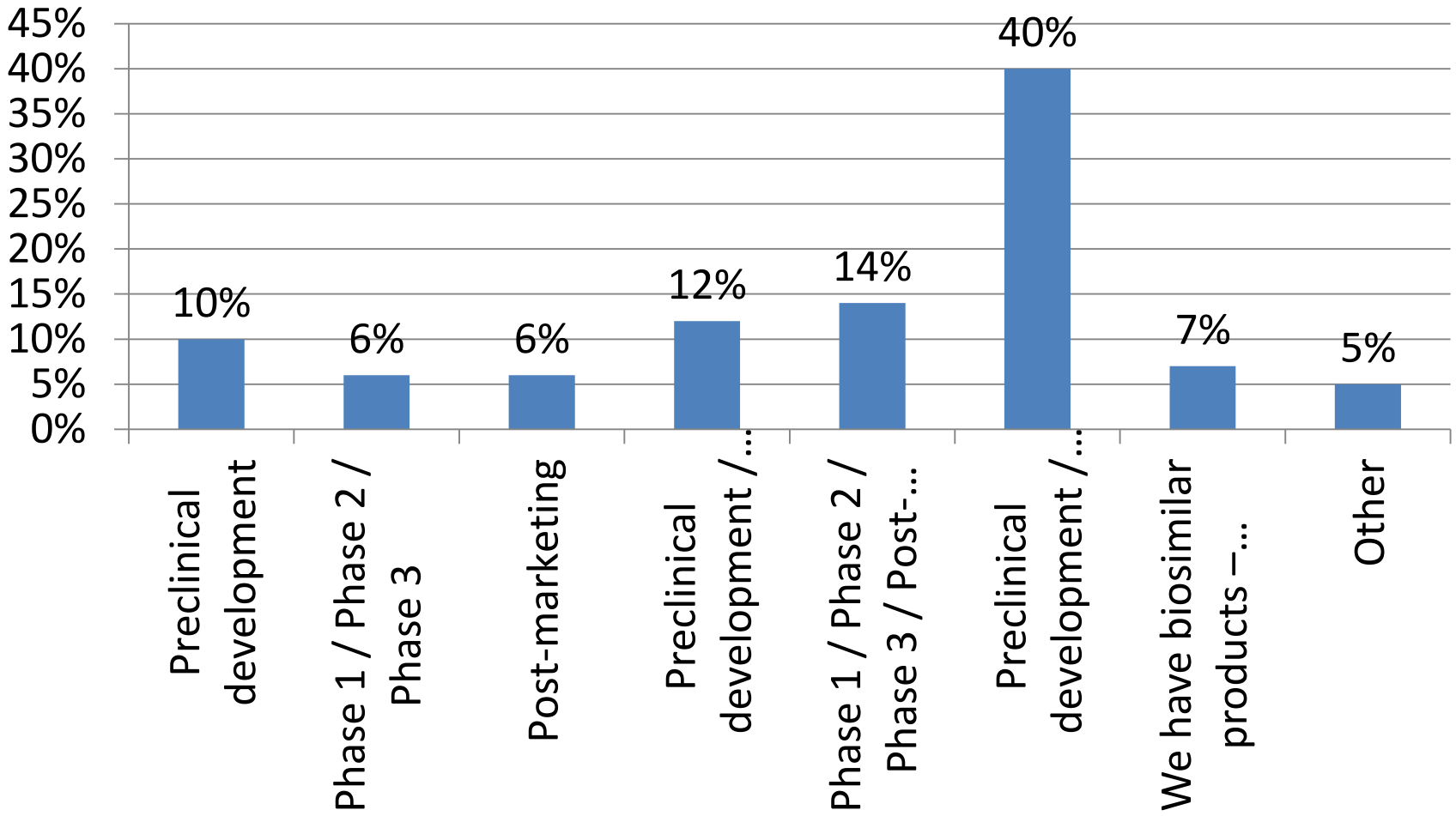
# Where are the bioassays developed?



# Stages at which assay(s) used

1. Preclinical development
2. Phase 1 / Phase 2 / Phase 3
3. Post-marketing
4. Preclinical development / Phase 1 / Phase 2 / Phase 3
5. Phase 1 / Phase 2 / Phase 3 / Post-marketing
6. Preclinical development / Phase 1 / Phase 2 / Phase 3 / Post-marketing
7. We have biosimilar products – therefore the above doesn't make sense
8. Other

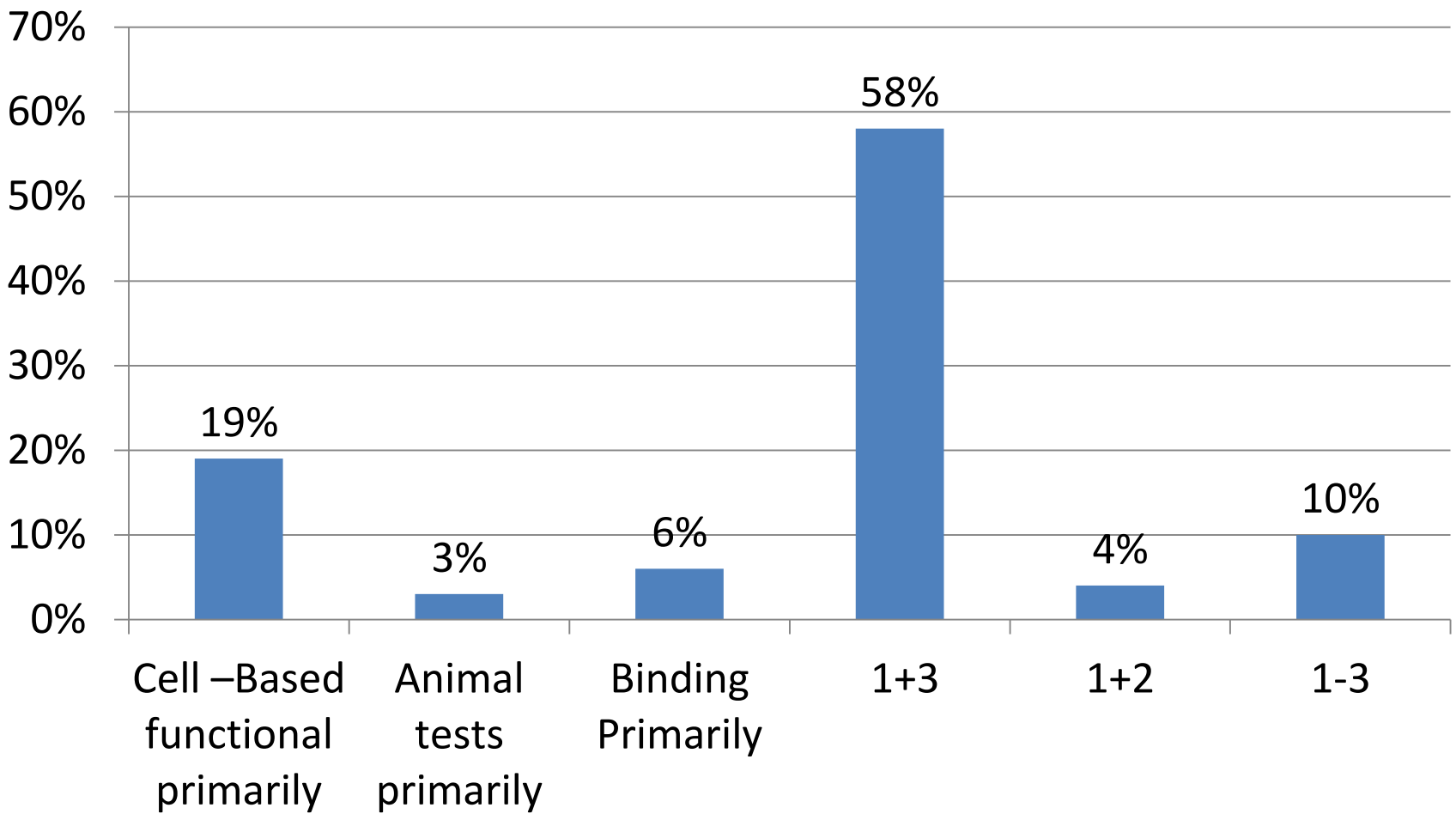
# Stages at which assay(s) used



# Functional or Ligand Binding?

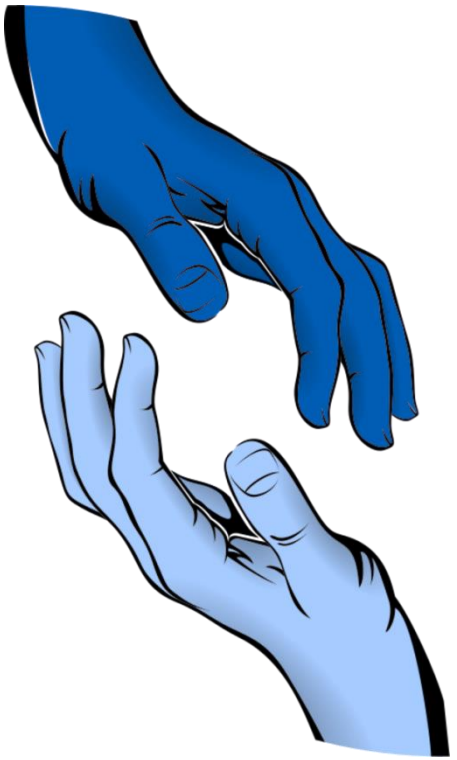
1. Cell –Based functional primarily
2. Animal tests primarily
3. Binding Primarily
4. 1+3
5. 1+2
6. 1-3

# Functional or Ligand Binding?



# Now on to the topic at Hand

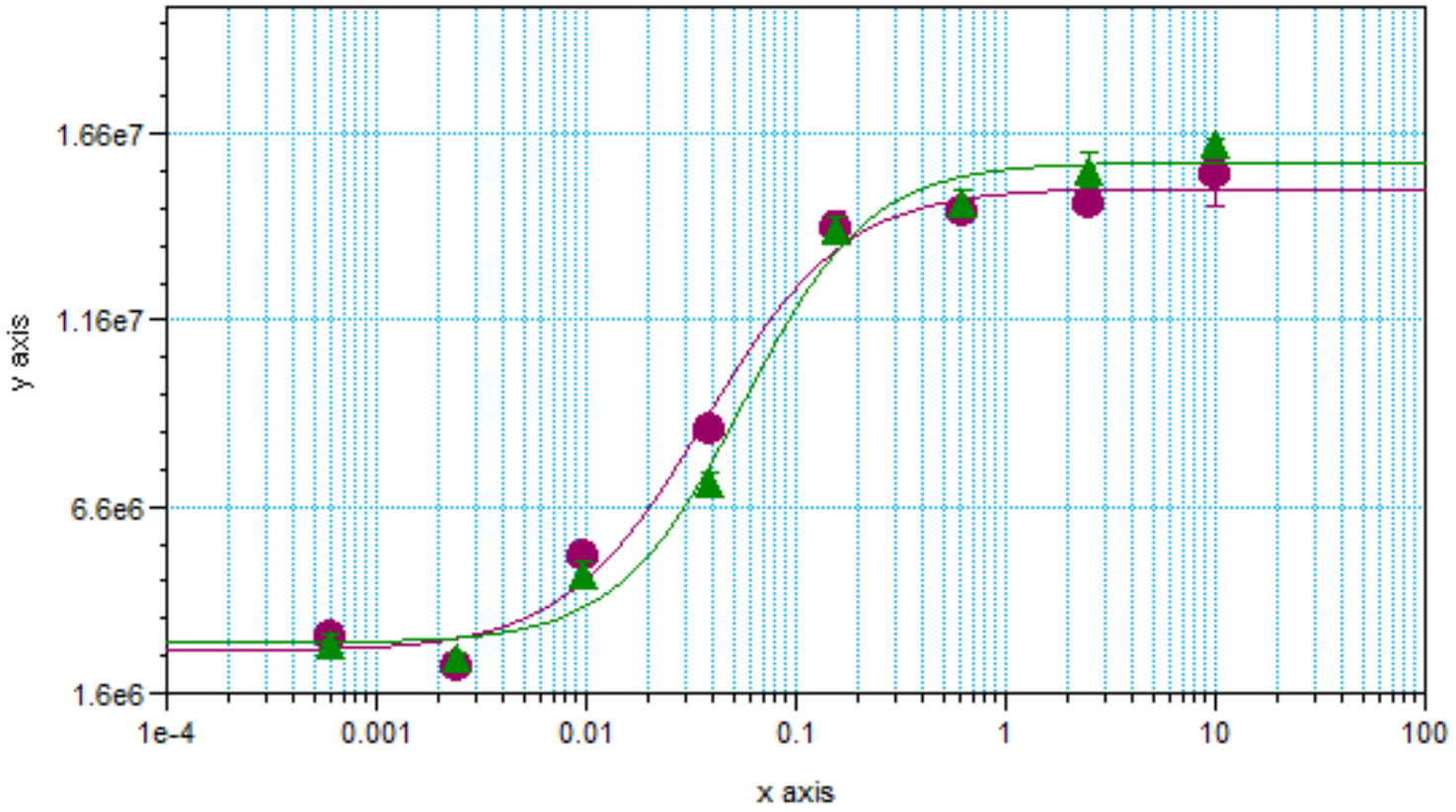
How Do We Combine the Results from Multiple Assay Runs into a single reportable result?



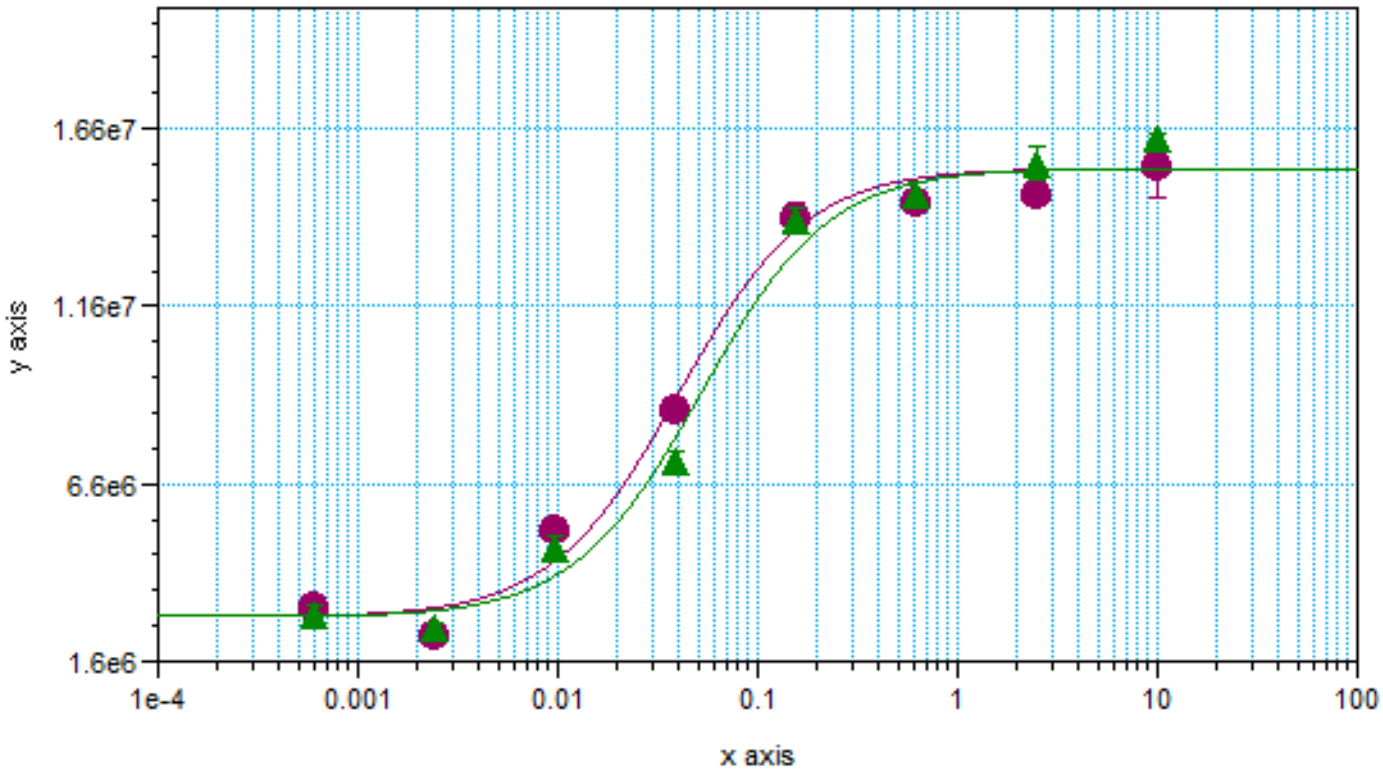
# Most of us Know How we get Potency Values off the Dose Response Curves

- Assuming it is a Cell Based Assay (or other in vitro method) fit with a 4 Parameter Logistic (4-PL) curve.
  - Typically we do a best fit 4-PL for each sample (test and reference)
  - Decide whether these best fit curves pass similarities (we have had lots of talks on the topic of similarity: difference vs. equivalence)
  - If these curves are deemed “similar” then a consensus curve is fitted (commone asymptotes and slope) and the ratio of the C parameters (aka the $ED_{50}$) equals RP

# Here is an Example of Best Fit



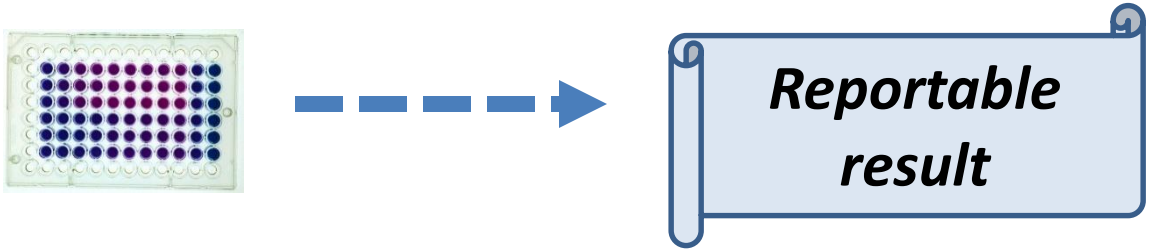
# Here is the Consensus Curve



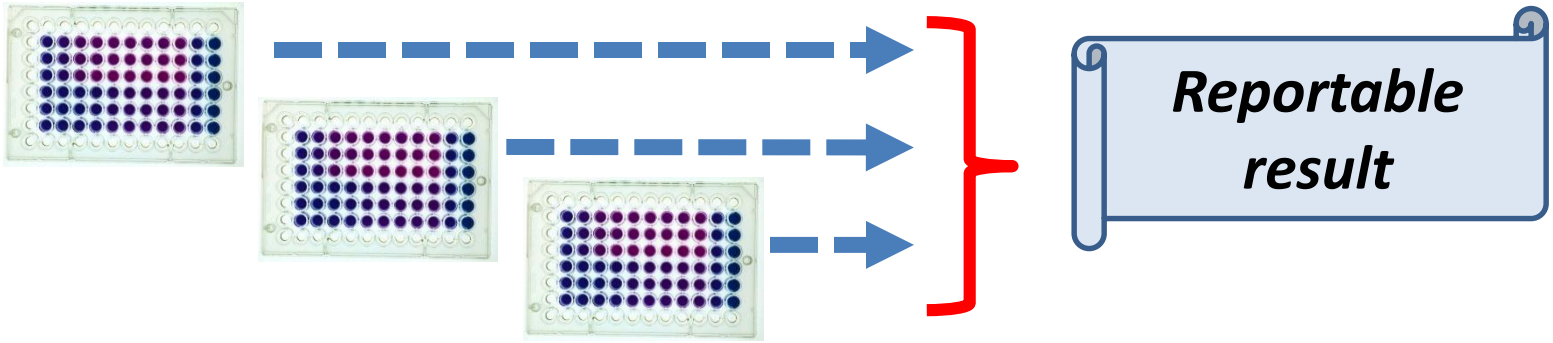

The ratio of the C parameters from these curves are reported as a potency value.

# Is this the Potency Value?

- What I have shown is a single plate.
- Is this the usual final reportable result?




- Or are we running multiple plates and combining the data in some fashion?

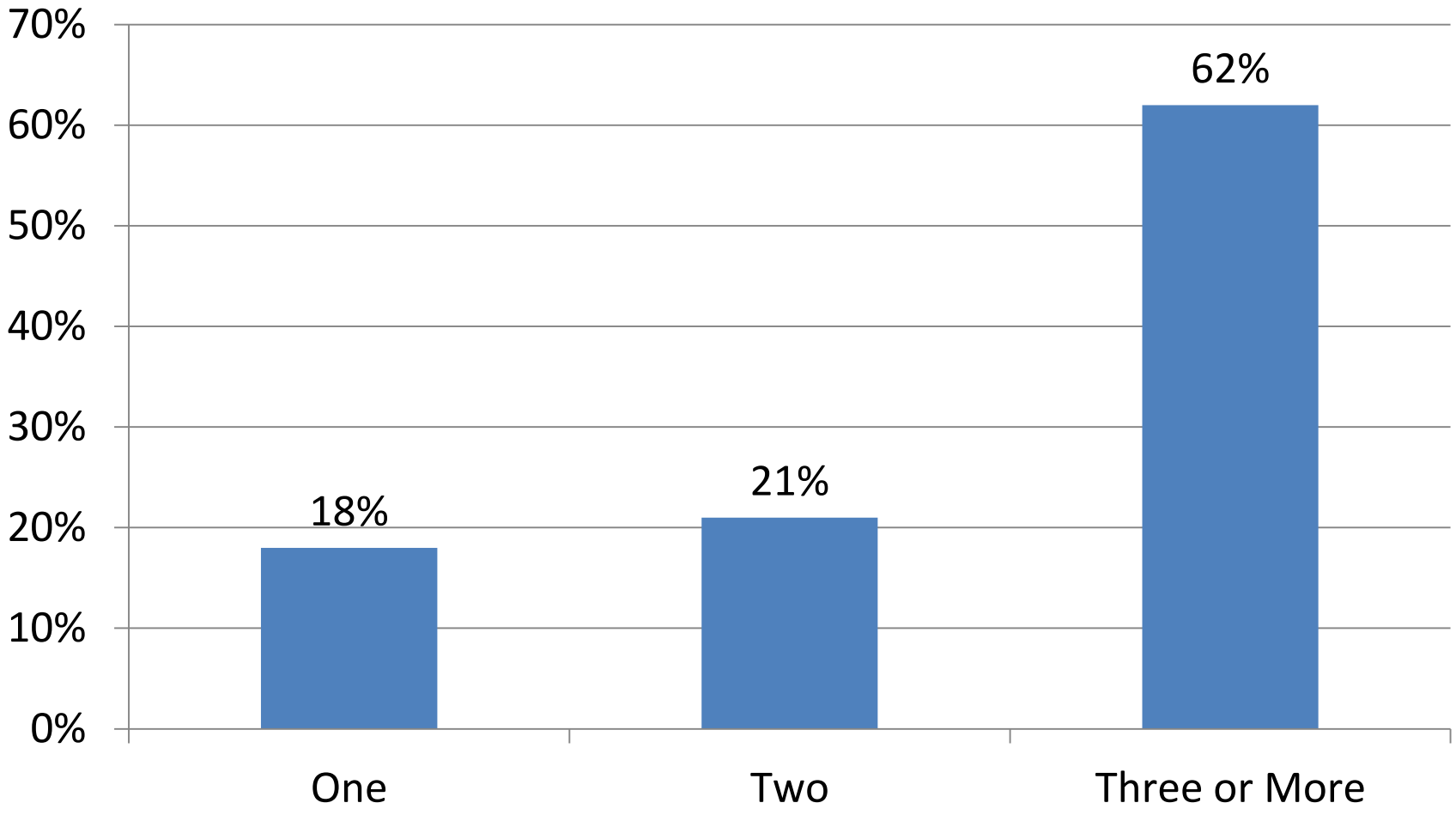


# How Many Runs?

How Many Plates do You Usually Run to Obtain a Reportable Potency Result?

1. One
2. Two
3. Three or More

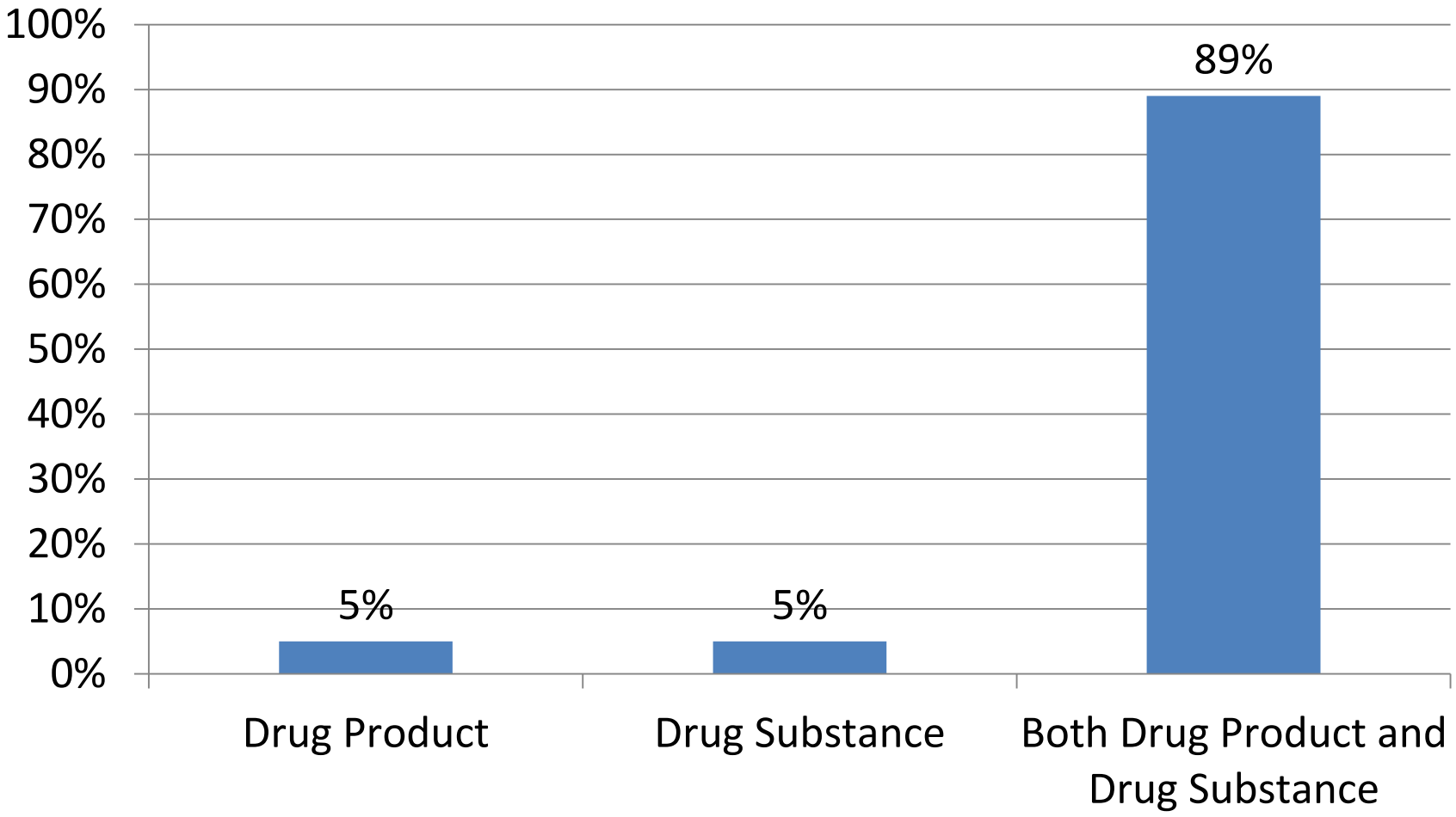
# How Many Plates do You Usually Run to Obtain a Reportable Potency Result?



# What Samples do you Run in Your Bioassay?

1. Drug Product
2. Drug Substance
3. Both Drug Product and Drug Substance

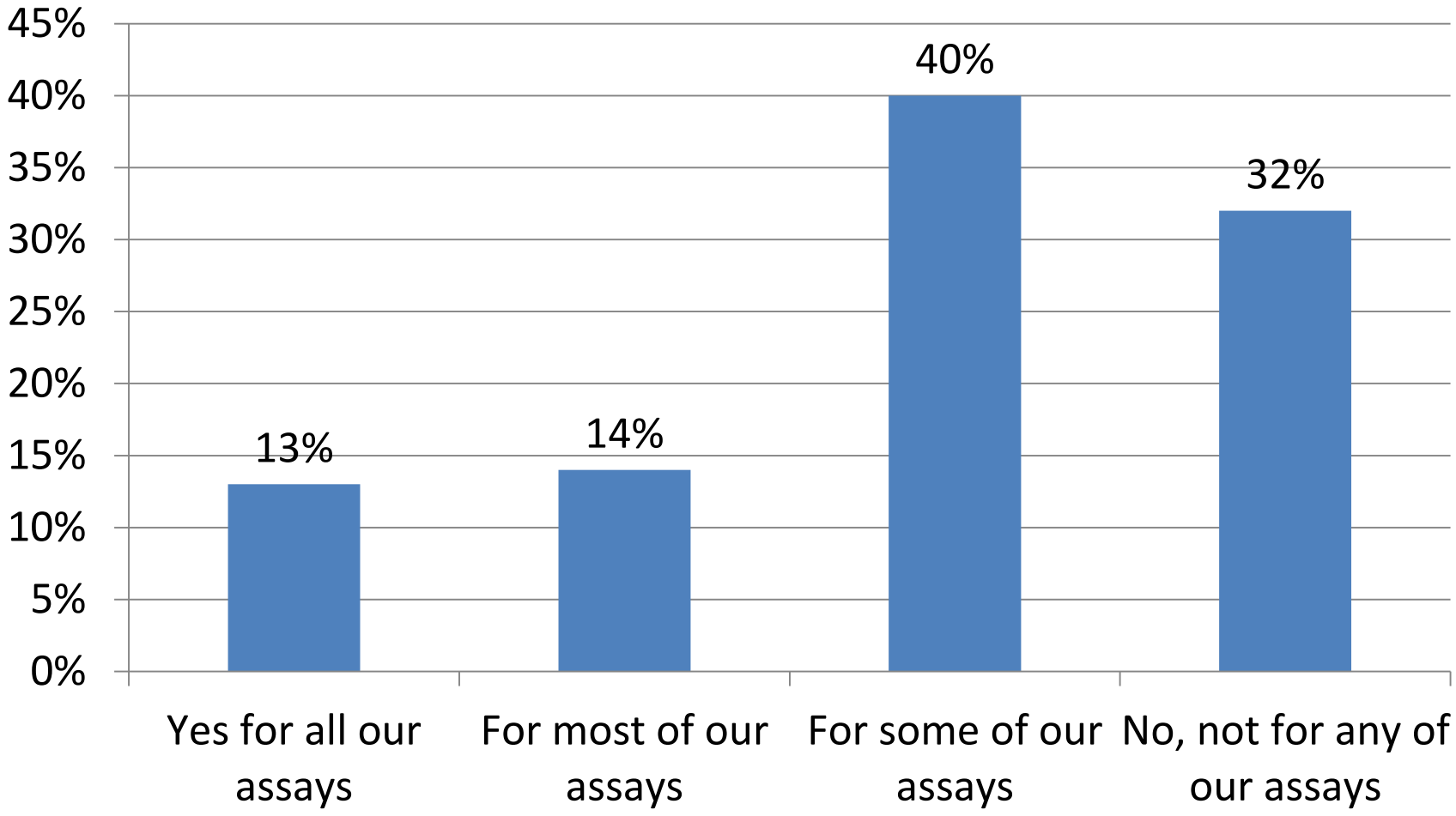
# What Samples do you Run in Your Bioassay?



# Has a Statistician Designed Your Approach for Combining Plate Data?

1. Yes for all our assays
2. For most of our assays
3. For some of our assays
4. No, not for any of our assays

# Has a Statistician Designed Your Approach for Combining Plate Data?



# If we run multiple plates…..

- How do we combine the results?
- It seems that there are many approaches.
- ***DISCLAIMER:*** I am only trying to get a discussion going on this. I have no preconceived notion about how this should be done. Or how it is typically being done. I would just like to hear more talks on the topic! So I am presenting some approaches and information…….

# What Do the USP and EP say?

- 1034 Section 4.1 Results for Multiple Assays
- Two Primary Questions to Ask
  - Are the assays mutually independent?
  - Are the results of the assays homogeneous?
- Depending on the answers they recommend the following:

# Simplest USP and EP Method

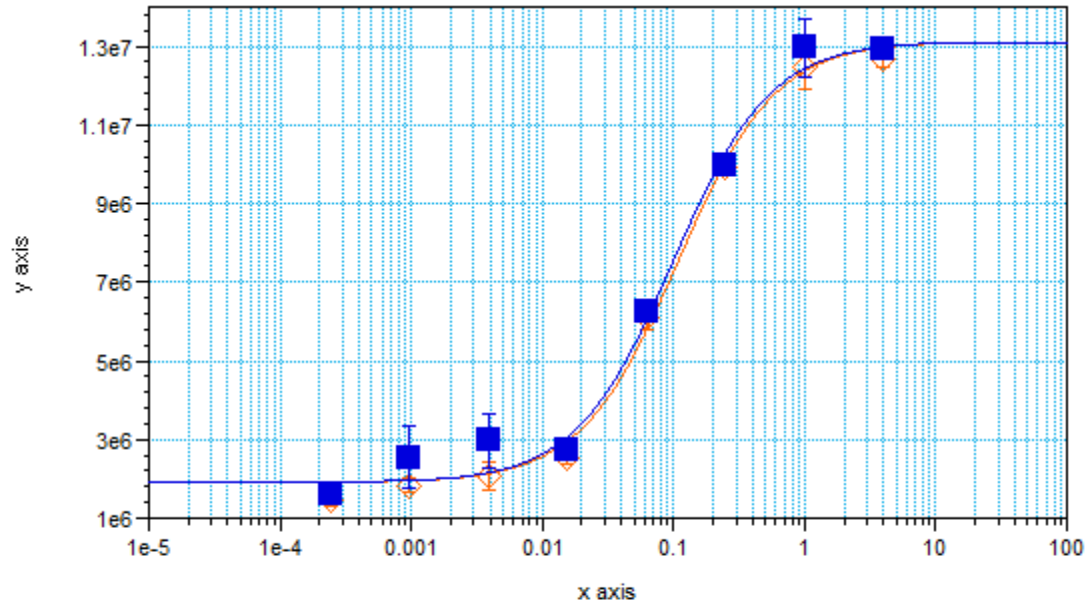
- Sample Based Interval Methods (Also known as the unweighted mean potency)

$$\text{Mean } \bar{R} = \sum_{i=1}^{N} R_i / N$$

$$\text{Standard Deviation } S = \sqrt{\frac{1}{N-1} \sum_{i=1}^{N} (R_i - \bar{R})^2}$$

$$\text{Standard Error } SE = S / \sqrt{N}$$

# Run 1 – Potency Calculation




| 4-P Fit: y = (A - D)/( 1 + (x/C)^B ) + D: | A | B | C | D | Rel. Pot. |
|---|---|---|---|---|---|
| RM (RM: concentration vs MeanValue) | 1.9e+06 | 1.17 | 0.108 | 1.31e+07 | 1 |
| QC (QC: Conc vs MeanValue) | 1.9e+06 | 1.17 | 0.099 | 1.31e+07 | 1.09 |

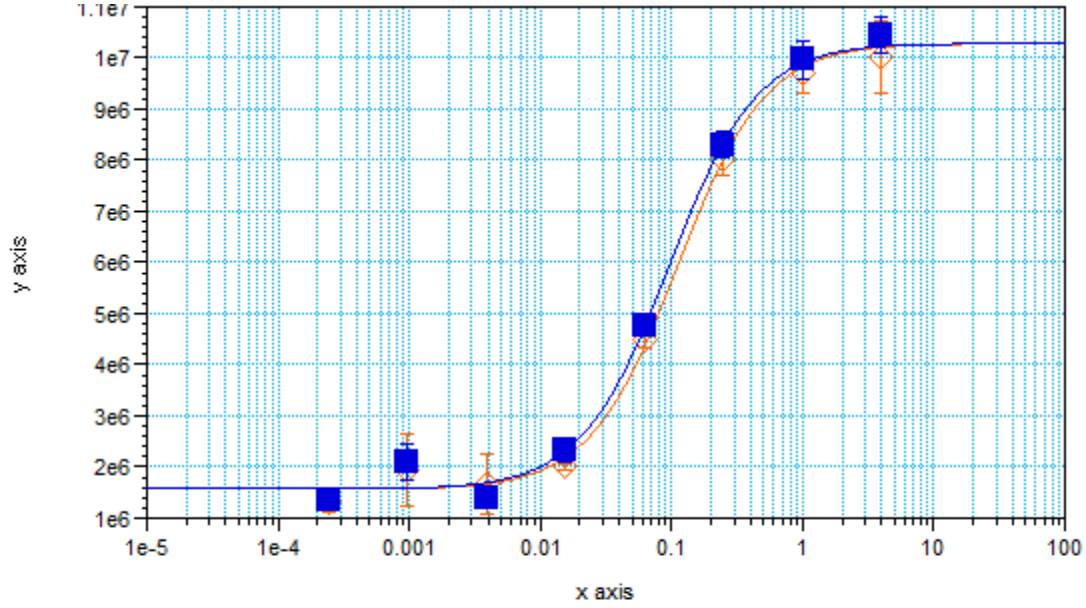
Weighting: Fixed

PLA (Std. Curve: RM) Degrees of Freedom: parallel = 11 free = 8 non-parallel = 3

R^2 = 0.993 F-stat = 0.882 F-prob = 0.49

Molecular Devices Read-out

# Run 2 - Potency Calculation




| 4-P Fit: y = (A - D)/( 1 + (x/C)^B ) + D: | A | B | C | D | Rel. Pot. |
|---|---|---|---|---|---|
| RM (RM: concentration vs MeanValue) | 1.56e+06 | 1.3 | 0.111 | 1.03e+07 | 1 |
| QC (QC: Conc vs MeanValue) | 1.56e+06 | 1.3 | 0.0959 | 1.03e+07 | 1.16 |

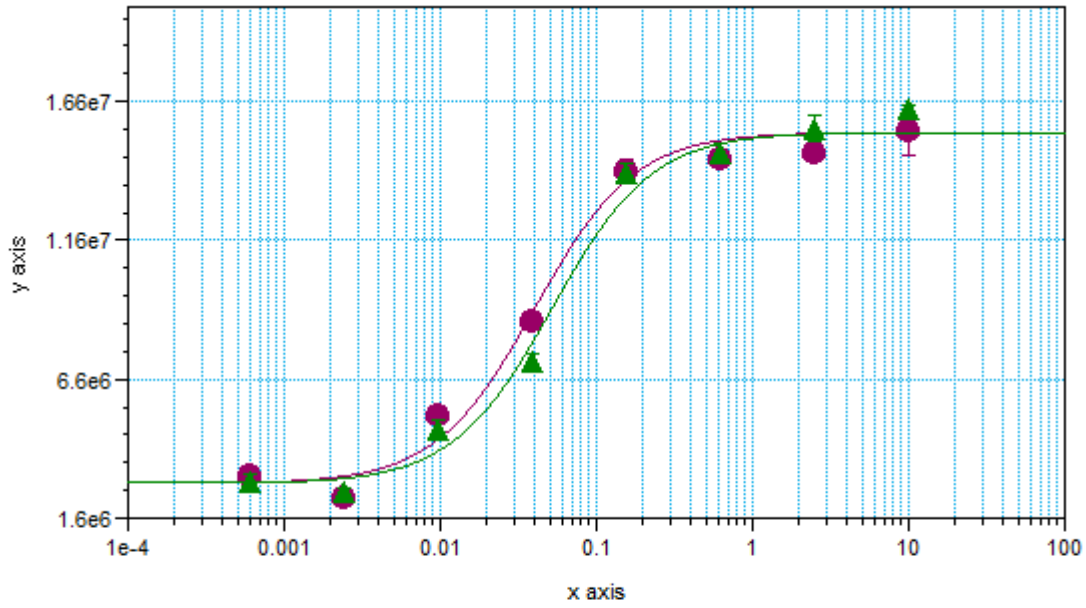
Weighting: Fixed

PLA (Std. Curve: RM) Degrees of Freedom: parallel = 11 free = 8 non-parallel = 3

R^2 = 0.996 F-stat = 0.44 F-prob = 0.73

Molecular Devices Read-out

# Run 3 – Potency Calculation




| 4-P Fit: y = (A - D)/( 1 + (x/C)^B ) + D: | A | B | C | D | Rel. Pot. |
|---|---|---|---|---|---|
| RM (RM: concentration vs MeanValue) | 2.83e+06 | 1.36 | 0.0403 | 1.55e+07 | 1 |
| QC (QC: concentration vs MeanValue) | 2.83e+06 | 1.36 | 0.052 | 1.55e+07 | 0.775 |

Weighting: Fixed

PLA (Std. Curve: RM) Degrees of Freedom: parallel = 11 free = 8 non-parallel = 3

R^2 = 0.988 F-stat = 0.57 F-prob = 0.65

Molecular Devices Output

# Simplest Approach

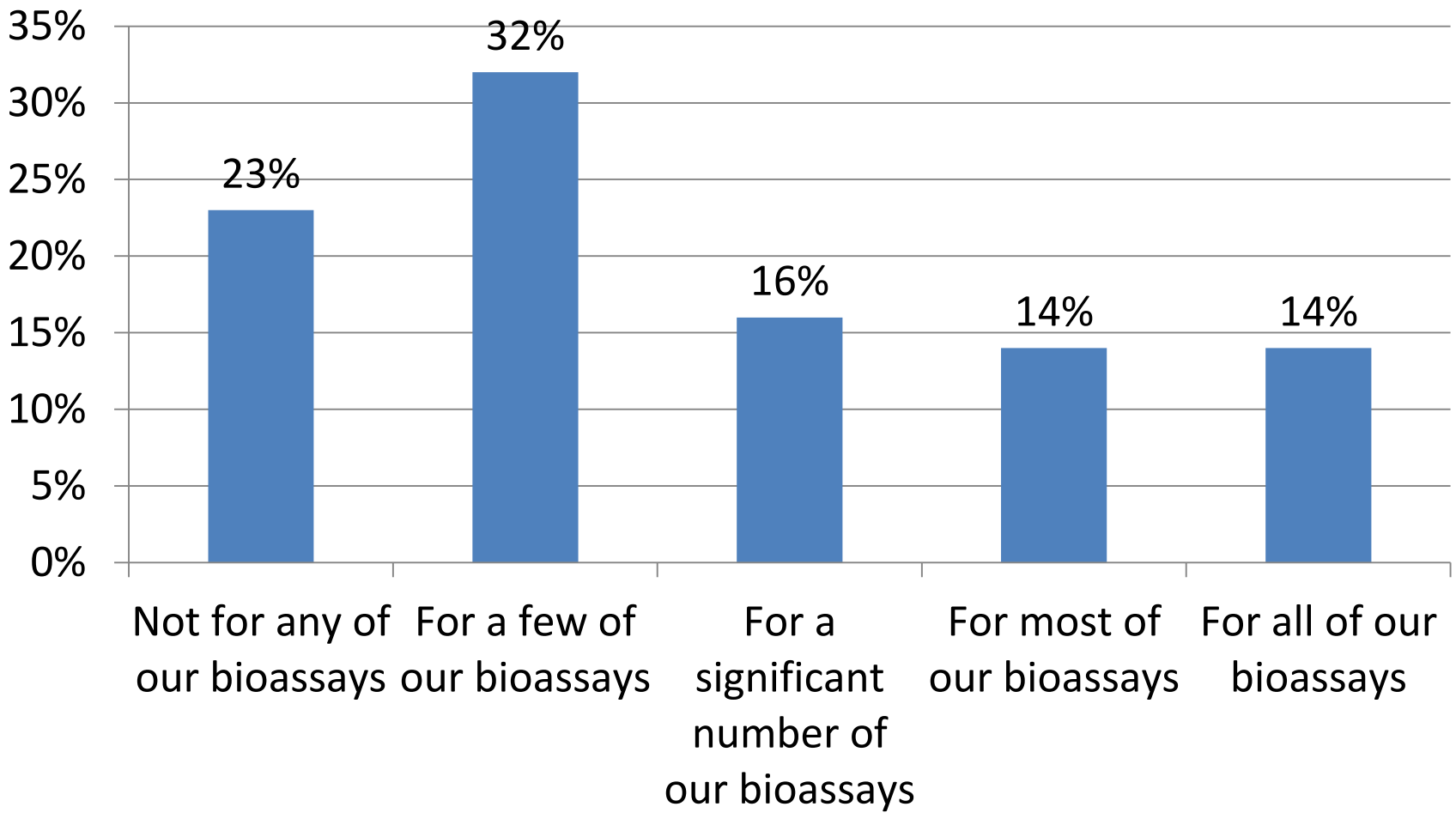
| | |
|---|---|
| Run 1 | 1.09 |
| Run 2 | 1.16 |
| Run 3 | 0.78 |
| | |
| Ave | 1.01 |
| Std. Dev | 0.21 |

**Reportable Value (RP) = *1.01  CI = (1.42 to 0.60)**

# Does Anyone Use this Method (Unweighted Mean Potency) for Any of their Reportable Value Calculation?

1. Not for any of our bioassays
2. For a few of our bioassays
3. For a significant number of our bioassays
4. For most of our bioassays
5. For all of our bioassays

# Does Anyone Use this Method (Unweighted Mean Potency) for Any of their Reportable Value Calculation?



# Compare Software for RP and CI for Unweighted Mean Potencies

| Software | Run 1 | Run 2 | Run 3 |
|---|---|---|---|
| **Stegmann** | 1.09122 (0.76556 to 1.5540) | 1.15899 (0.90417 to 1.48563) | 0.77495 (0.49572 to 1.21148) |
| **Unistat** | 1.0912 (0.8877 to 1.3414) | 1.1590 (0.9593 to 1.4003) | 0.7750 (0.6210 to 0.9657) |
| **Molecular Devices** | 1.09 | 1.16 | 0.775 |

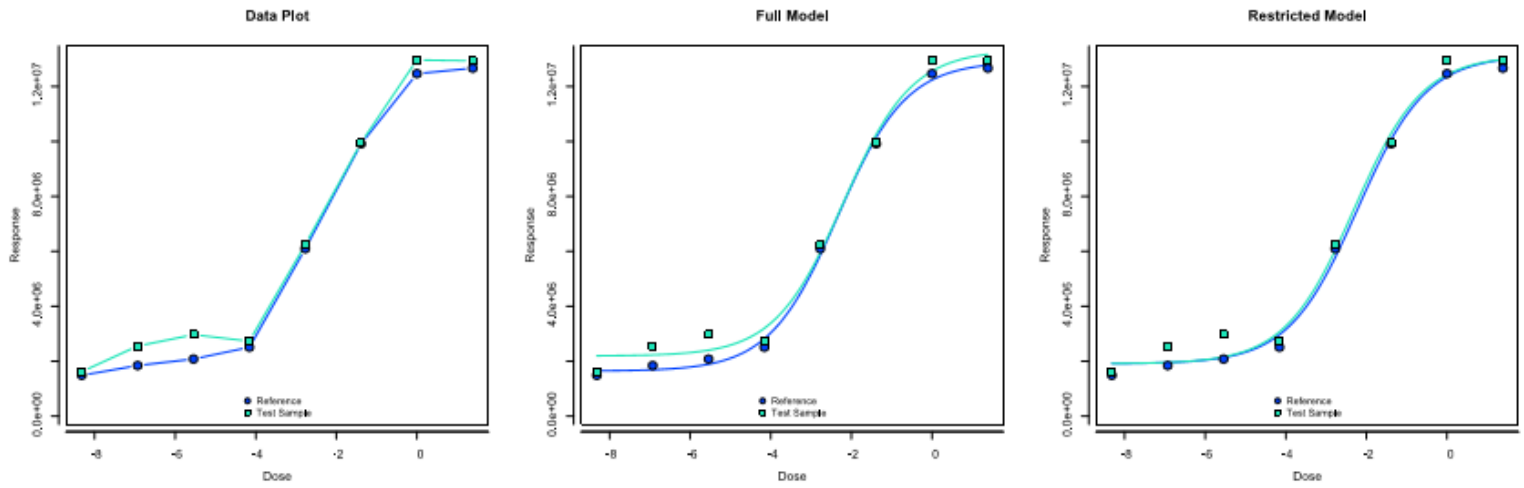
Although Similar they are different…..Why?

# Run 1 Means

## Potency Estimation

| Relative Potency | Test Sample | Reference |
|---|---|---|
| Potency Ratio | 1.09122 | |
| 95% Confidence Interval | 0.76556 - 1.55540 | |
| Relative Confidence Interval | 70.16% - 142.54% (72.38%) | |

## Graphics



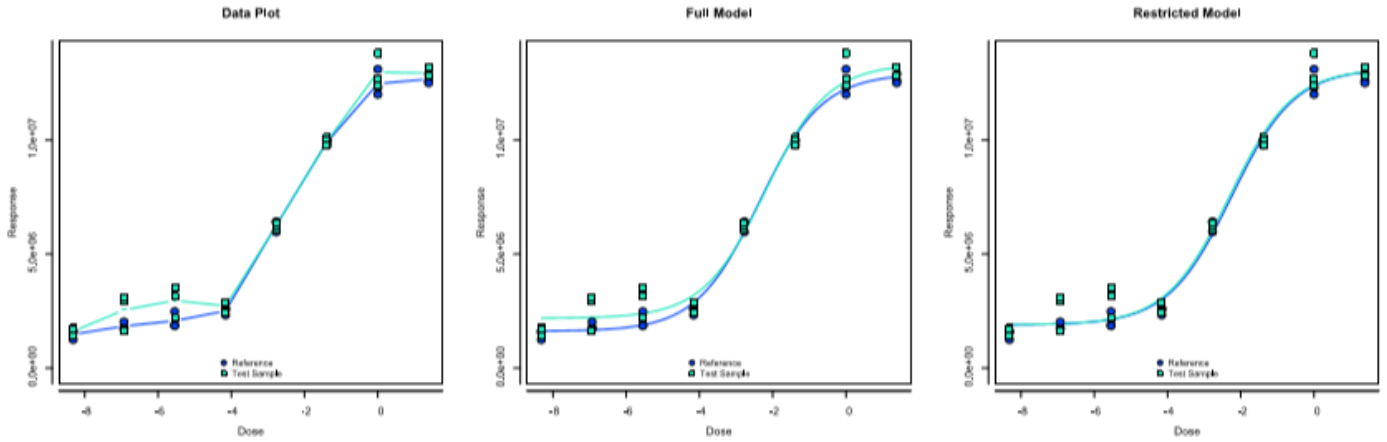

The above values were calculated from the example case on Stegmann Systems PLA. Calculation performed by Mattias Schmitt . Contact information: matthias.schmitt@stegmannsystems.com . All the brilliance is him…any errors are mine. AND he is here at the meeting…..so find him and ask him all the questions!!!!

# Run 1 - Replicates

**Potency Estimation**

| Relative Potency | Test Sample | Reference |
|---|---|---|
| Potency Ratio | 1.06121 | |
| 95% Confidence Interval | 0.90675 - 1.24199 | |
| Relative Confidence Interval | 85.44% - 117.04% (31.59%) | |

**Graphics**

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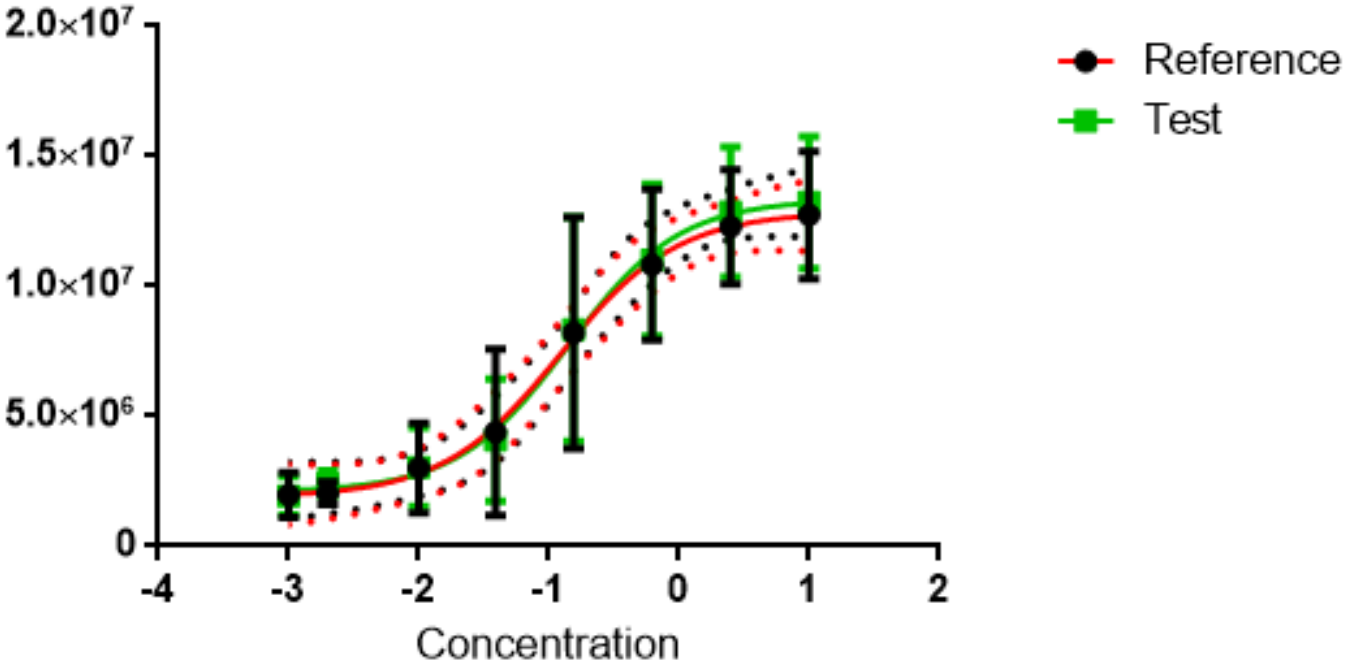
# Differing Values: Mostly in the confidence intervals

- I am actually impressed that the overall values were so close between the three software programs.
- The confidence interval seems to be very dependent of the method of data analysis chosen…..
  - Lots of choices we have to make as the scientists. Means, replicates, outlier analysis, data transformation, etc.
  - Beyond the scope of this talk…..but if someone in the audience would like to have an entire talk about this next year…..come and volunteer!!!

# Another Path I have Seen (Not in either the USP or the EP)

- Take all of the data from all of the runs
- Put them into a single analysis as if they were from a single plate
- Have the software calculate the ED50 shift and the Confidence Interval of the ratio.

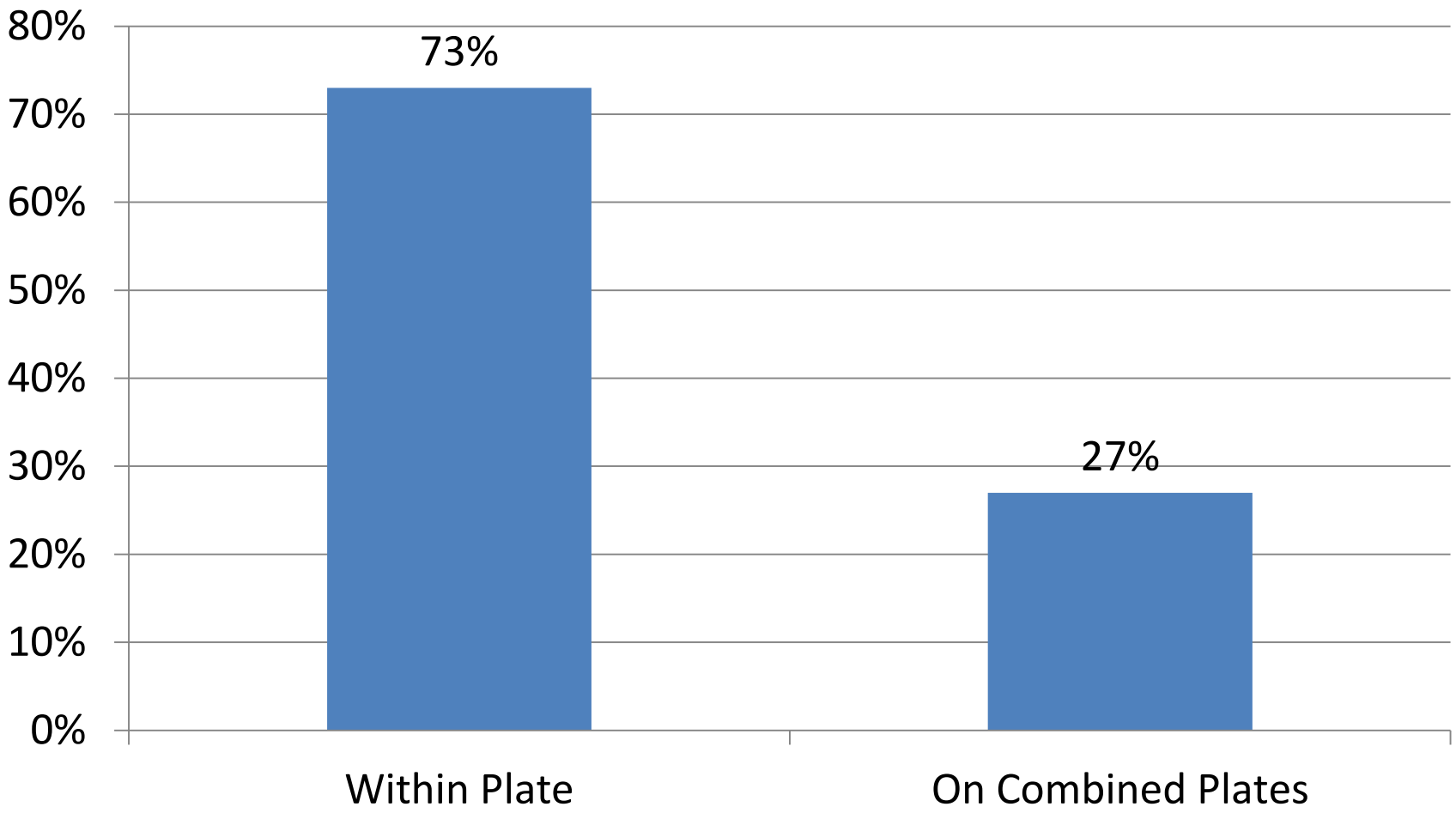
# Putting All Data on a Single Plot (Best Fit)



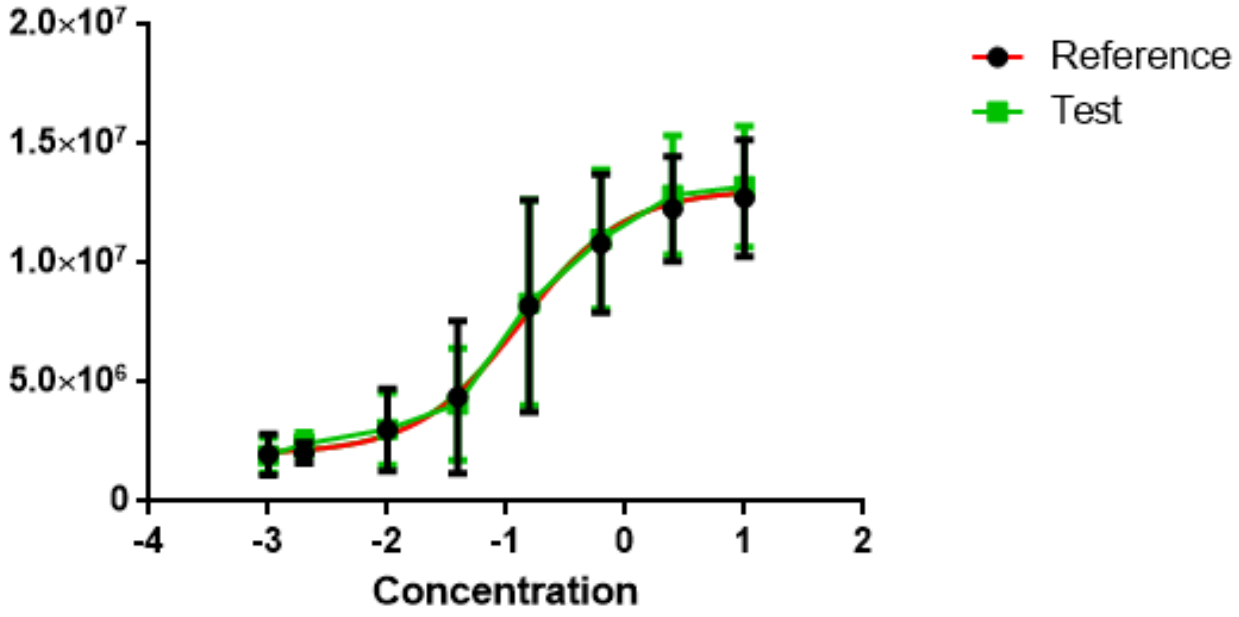
# When do You Assess Similarity?

1. Within Plate
2. On Combined Plates

# When do You Assess Similarity



# Reportable Value Using Constrained Curves




**Reportable Value RP = 0.9635 CI = (1.534 to 0.3926)**

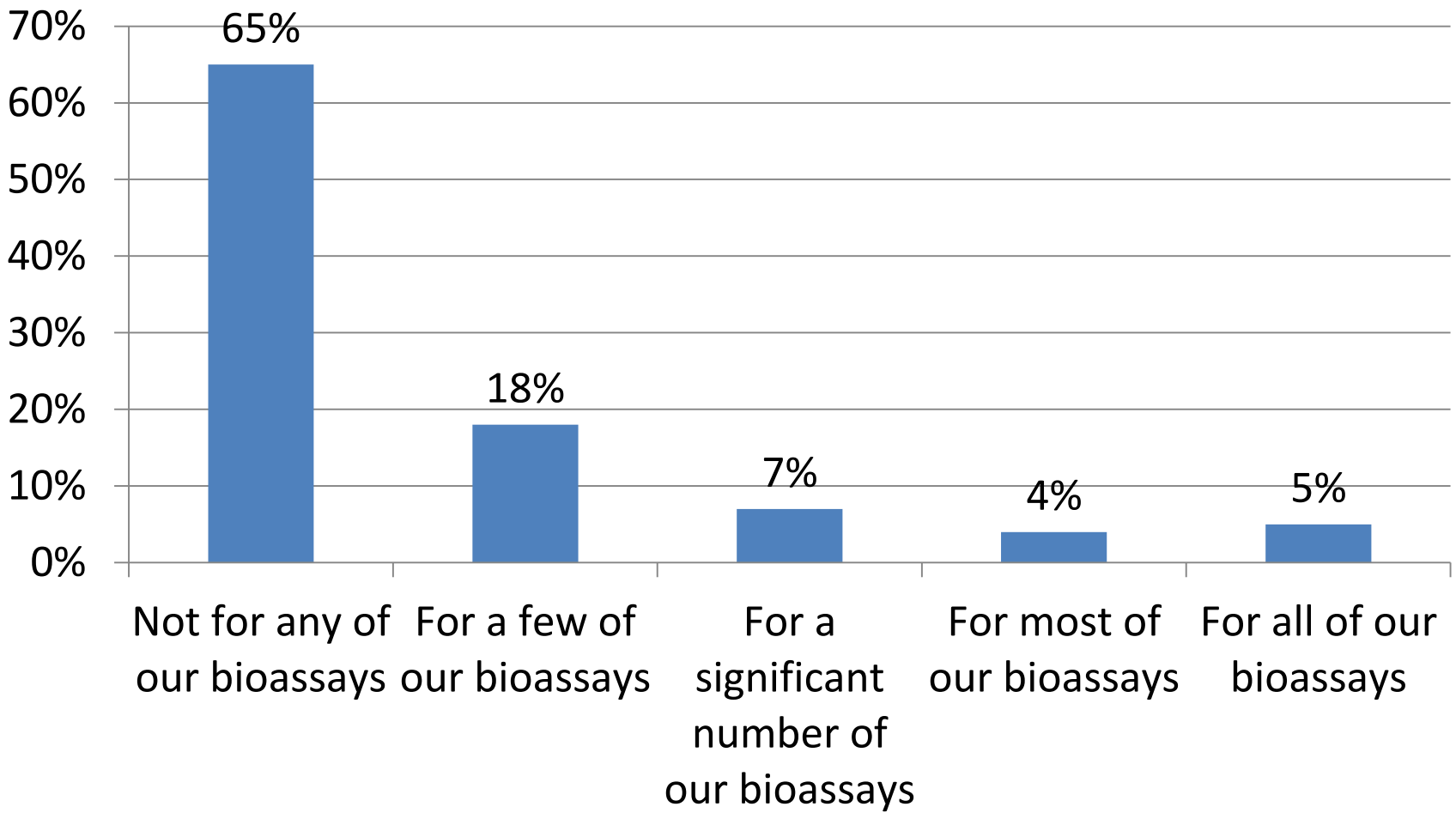
Compare this to Prior Method value:

Reportable Value RP = 1.01 CI =(1.42 to 0.60)

# Does Anyone Use this Method (Single Graph) for Any of their Reportable Value Calculation?

1. Not for any of our bioassays
2. For a few of our bioassays
3. For a significant number of our bioassays
4. For most of our bioassays
5. For all of our bioassays

# Does Anyone Use this Method (Single Graph) for Any of their Reportable Value Calculation?



# Please Note

- In this example one of the plates had very different dose-response curve characteristics. This made the Confidence Interval of the ratio very large for the approach #2
- If the response is fairly stable from plate to plate this might be a more viable approach

# Back to the USP/EP

- Homogeneously Weighted Combination
- Heterogeneously Weighted Combination according to European Pharmacopoeia, chapter 5.3
- Heterogeneously Weighted Combination according to US Pharmacopeia <111>
- Heterogeneously Weighted Combination according to US Pharmacopeia <1034>

# Individual Potency Values

***Combination of Assays***

***Data***

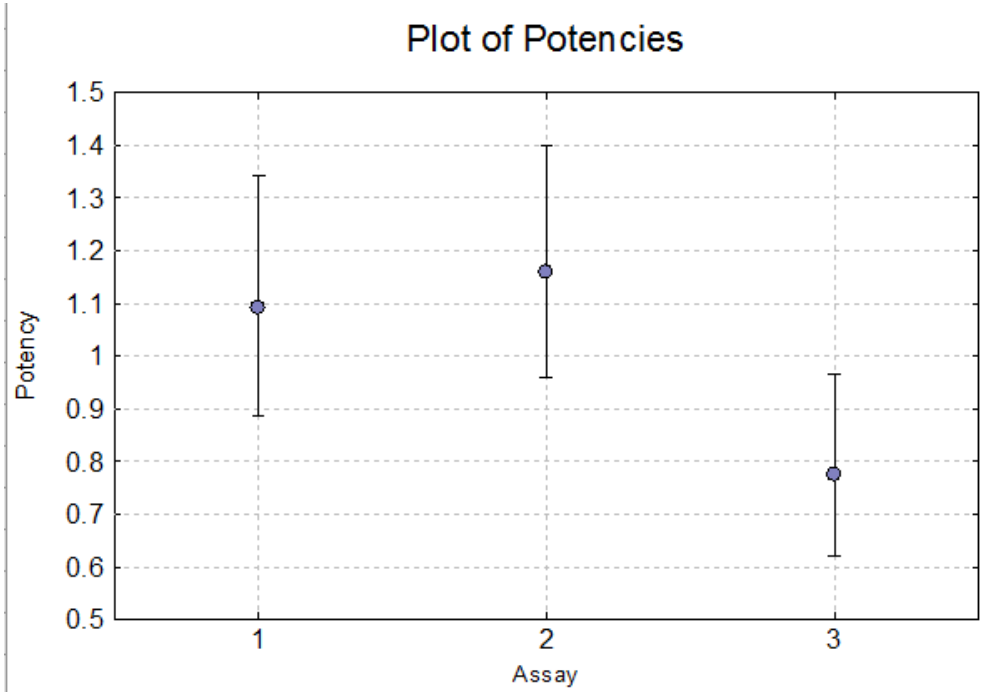
| Assay | Potency | Lower 95% | Upper 95% | DoF |
|---|---|---|---|---|
| 1 | 1.0912 | 0.8877 | 1.3414 | 43 |
| 2 | 1.1590 | 0.9593 | 1.4003 | 43 |
| 3 | 0.7750 | 0.6219 | 0.9657 | 43 |

The above values were calculated from the example case on Unistat. Calculation performed by Mat Toker from Unistat. Contact information: unistat@unistat.com. All the brilliance is him…any errors are mine.

# WHY USE WEIGHTED METHODS FOR COMBINING DATA?

Lets take a look at the example case again…….

# Why Weighted Means?

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# USP is Similar but…..

- It differs in how it defines/calculates the confidence interval…..
- Ask your Statistician about this if you are interested……

## Relative Potency

### Combination Calculation of Relative Potencies

| Sample | Relative Potency | Lower Limit | Upper Limit | *W* | *df* |
|---|---|---|---|---|---|
| 1 | 1.09122 | 0.76556 | 1.55540 | 42.32916 | 8 |
| 2 | 1.15899 | 0.90417 | 1.48563 | 86.25797 | 8 |
| 3 | 0.77495 | 0.49572 | 1.21148 | 26.63849 | 8 |

### Tests on Combination Result

| Homogeneity Test | | |
|---|---|---|
| $\chi^2$<br>$\chi^2_{critical}$ (95.00%)<br>*df* | 3.33443<br>5.99146<br>2 | Homogeneous |

| | Passed | Failed (Rejected) | Failed (Warning) | Passed (Info) | Not Calculated |
|---|---|---|---|---|---|
| Suitability | 0 | 0 | 0 | 0 | 0 |

### Calculation of the Combined Relative Potency and 95% Confidence Limits

| | Results |
|---|---|
| Selected Method | Homogeneously weighted |
| Combined Potency | 1.06401 |
| Confidence Interval | 0.90158 - 1.25571 |
| Combination of Assay Results State | NO TESTS AVAILABLE |

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# Heterogeneously Weighted Combination according to European Pharmacopoeia, chapter 5.3

- The variance of the heterogeneity between assays is calculates as:
- $$v = \frac{\sum M_i^2 - \frac{1}{n}\left(\sum M_i\right)^2}{n-1}$$
- where: $V_i = \frac{1}{W_i}$
- A semi weight is then defined as: $W_i' = \frac{1}{(V_i + v)}$
- The semi weighted mean potency and its confidence interval is then calculated as in the Weighted Mean Potency.

https://www.unistat.com/guide/bioassay-analysis-combination-of-assays/

# USP again is very similar

- Subtracts a term on the weighting calculation.
- Again if you are interested ask your favorite statistician for an explanation……

# Results from These Approaches

***Combined Potency EP***

| Geometric Mean | Potency | Lower 95% | Upper 95% |
|---|---|---|---|
| Weighted EP | 1.0127 | 0.9022 | 1.1368 |
| Semi-weighted EP | 1.0011 | 0.8309 | 1.2061 |
| Unweighted | 0.9933 | 0.5793 | 1.7034 |

***Combined Potency USP***

| Geometric Mean | Potency | Lower 95% | Upper 95% |
|---|---|---|---|
| Weighted USP | 1.0127 | 0.8989 | 1.1410 |
| Semi-weighted USP | 0.9976 | 0.7786 | 1.2783 |
| Unweighted | 0.9933 | 0.5793 | 1.7034 |

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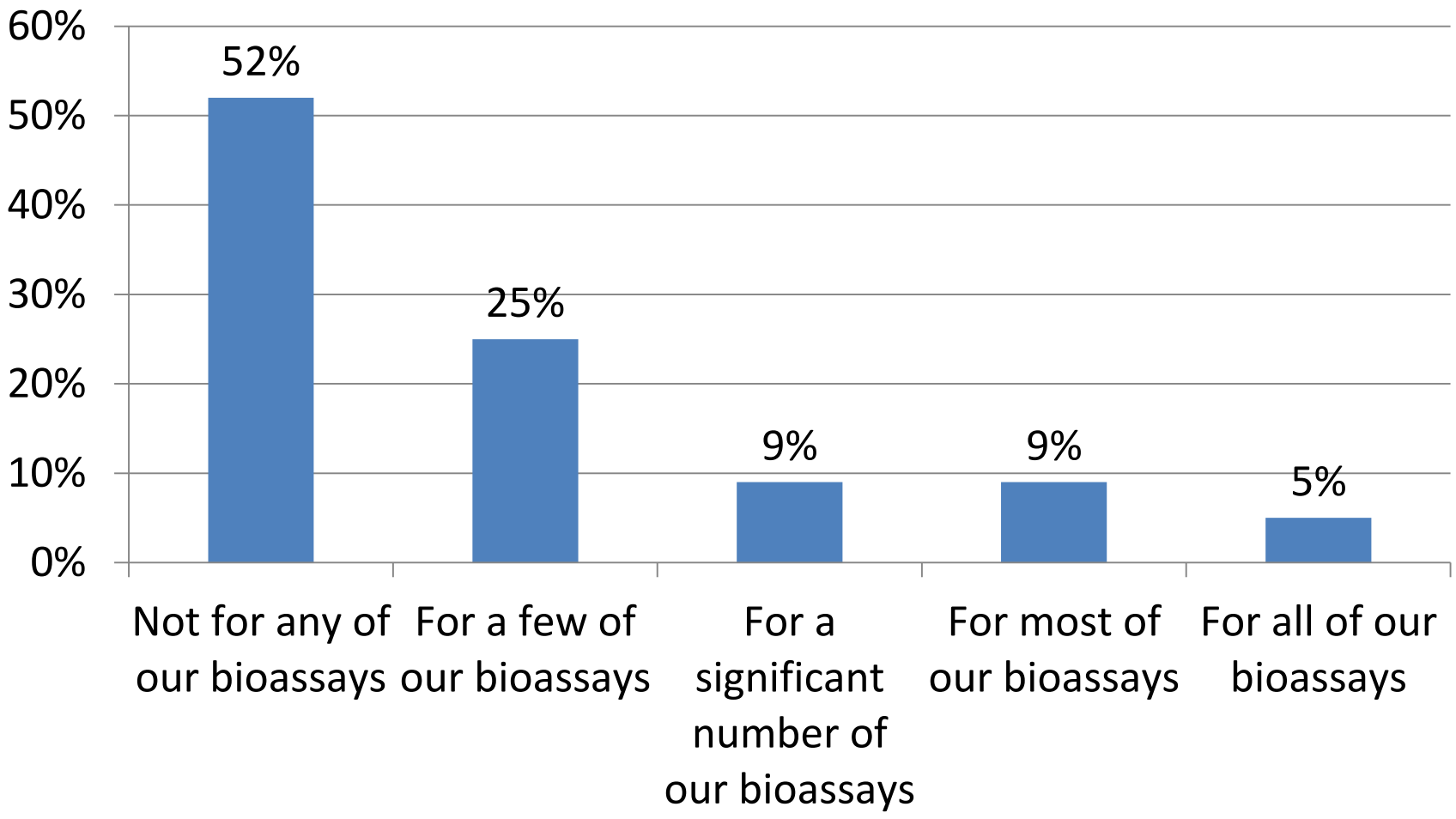

# Thank you for Answering These Questions.

- Survey Results will be posted on the web page.
- Please if you are interested in presenting on this topic, contact me at:
  - Laureen.Little@bebpa.org
  - Or submit an abstract directly to our website at www.bebpa.org

  - And now…….the fun begins……Gala information