# Speeding up assay development with automated workflow for assessing plate bias of microtiter plate assays

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UK JMP Users Group Meeting

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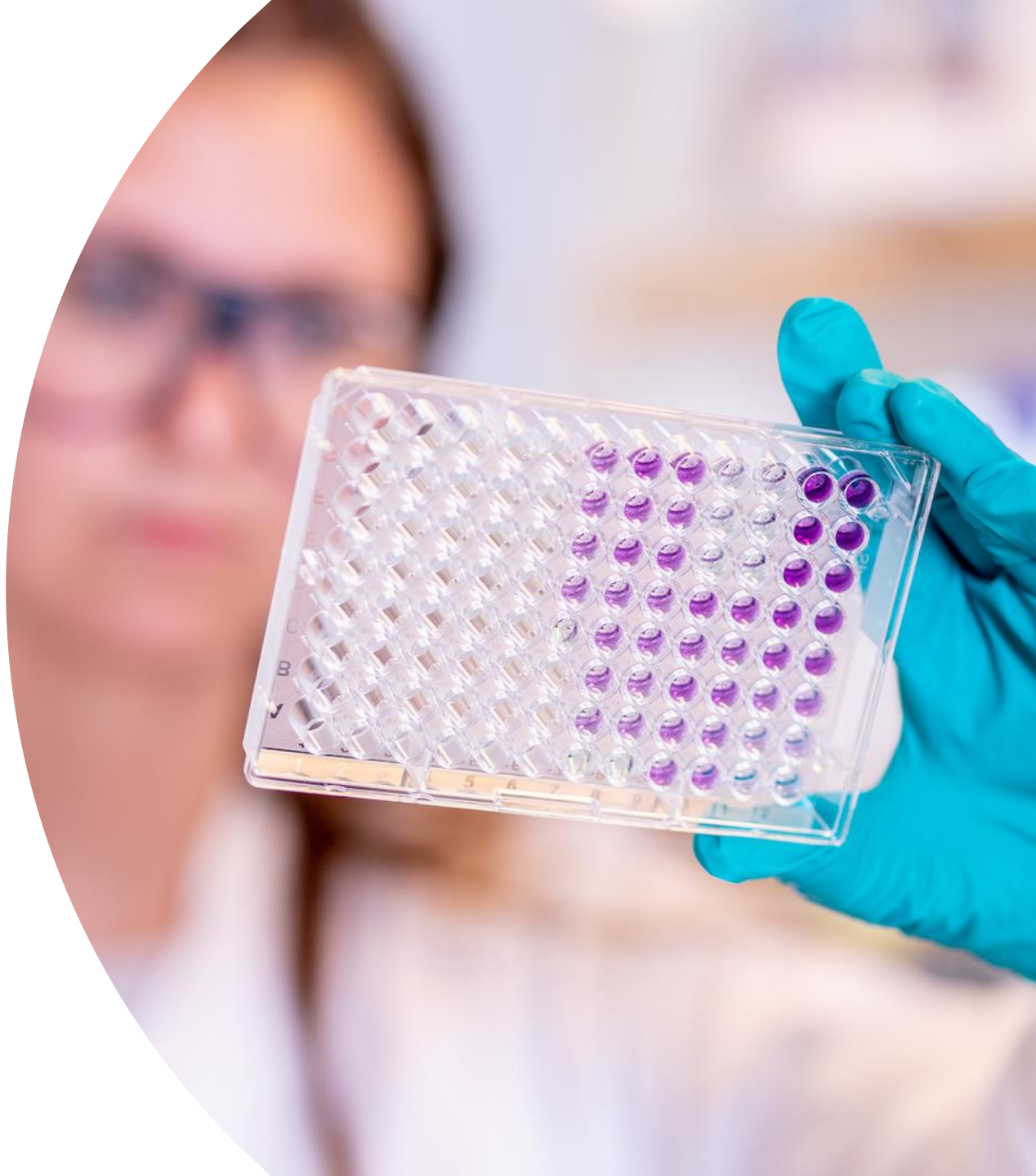


# Acknowledgements

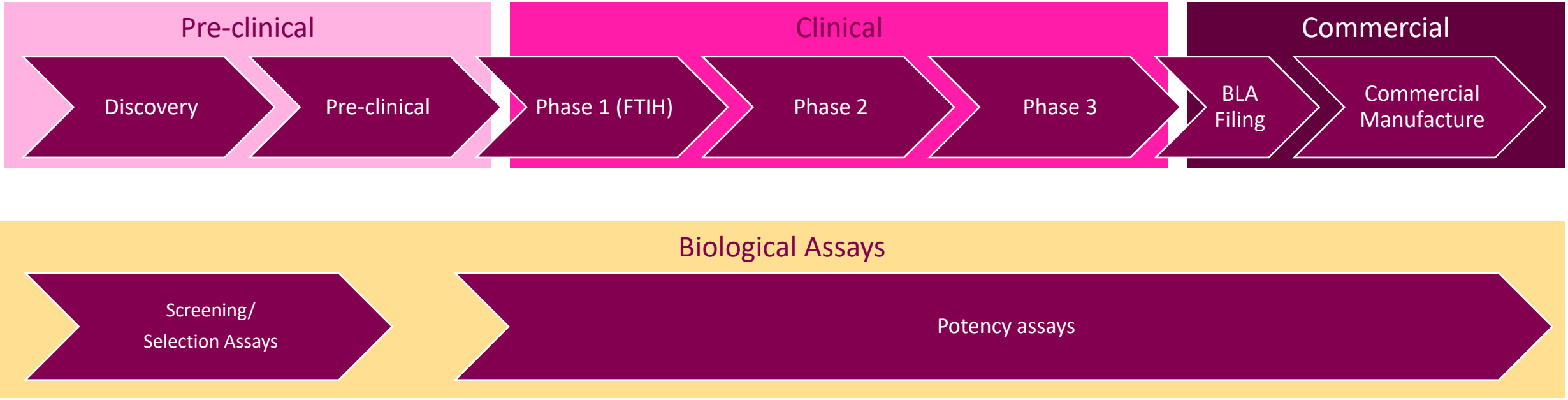
Biopharmaceutical Development, AstraZeneca:

- Deepika Srivastava
- Barbara Hebeis

# Presentation outline

1. Potency testing of biologics
2. Using microtiter plates in bioassays
3. Plate bias and uniformity
4. JMP demo

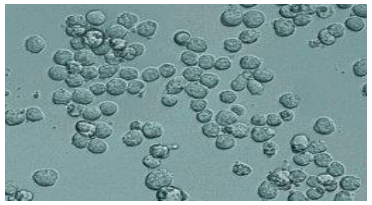
# Putting Potency Assays into Drug R&D Perspective



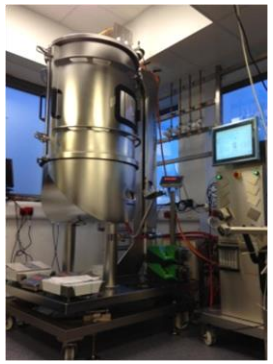

Which molecule has biological activity?

Is the biological activity at a similar level between lots? (Lot-to-lot consistency)

# Potency assays as part of a typical biopharmaceutical manufacturing process



Cell culture



Bioreactor process

Clarification & purification

Formulation & packaging

Finding optimal purification & formulation

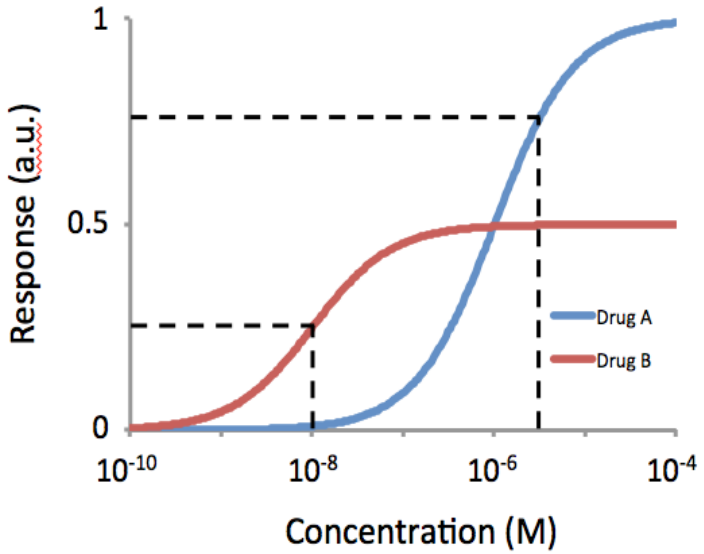
Enabling optimisation of manufacturing process

Enabling optimisation of manufacturing process

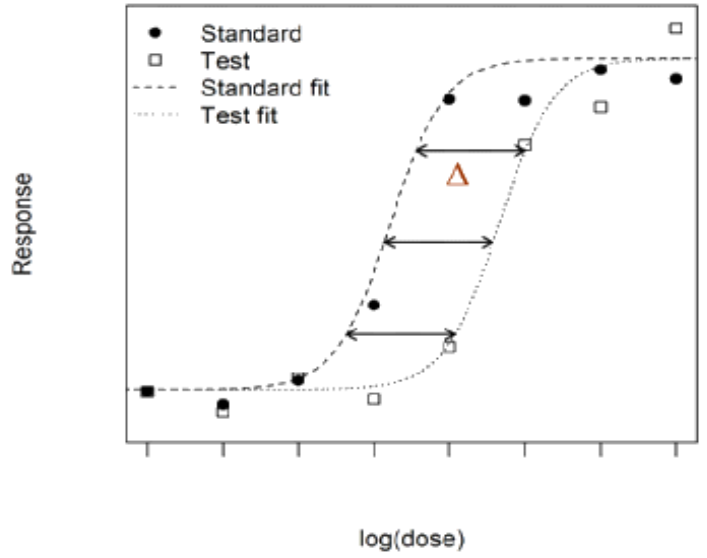
**Analytical testing for release & characterisation**

- Potency testing is a critical property of the drug and therefore is part of the formal control system within CMC (Chemistry, Manufacturing Process and Controls)

# Screening vs potency assays - measuring biological activity




KIbrain, CC BY-SA 4.0 https://creativecommons.org/licenses/by-sa/4.0, via Wikimedia Commons



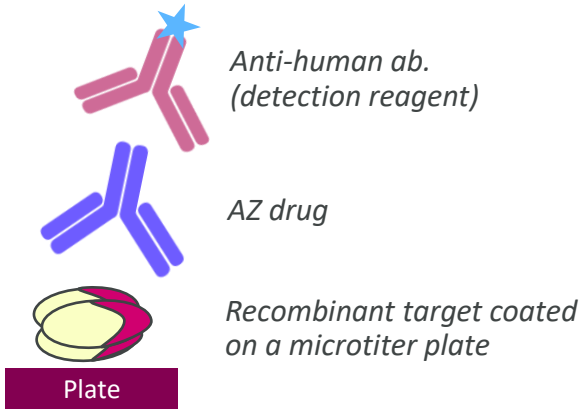

https://www.quantics.co.uk/qubas-bioassay-software2/qubashelp/relative_potency_1.htm

**Screening/Selection Bioassays:**
Resolve log scale differences between
**different drugs**

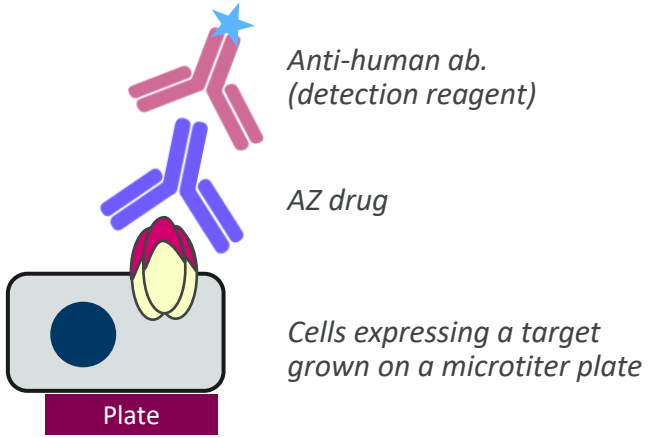
**CMC Potency Assays:**
Resolve within 2 fold change between
**different lots of the same drug**

# Common types of potency assays

## Non cell-based target binding assays

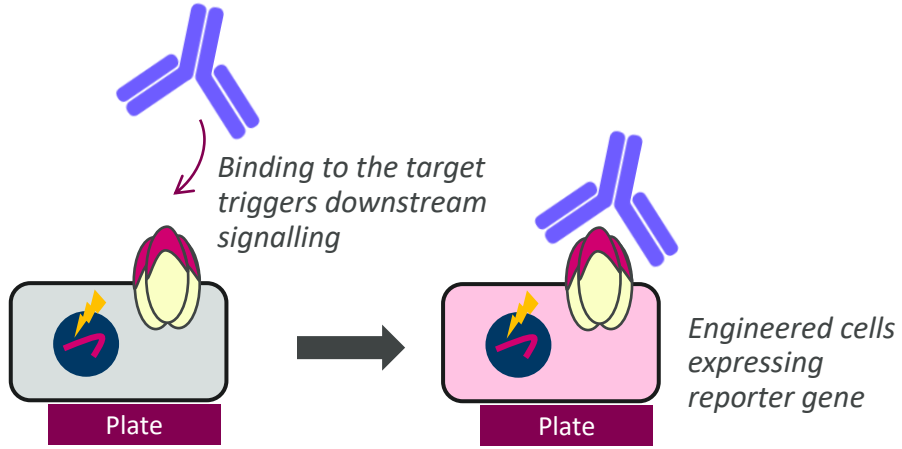



## Cell-based target binding assays



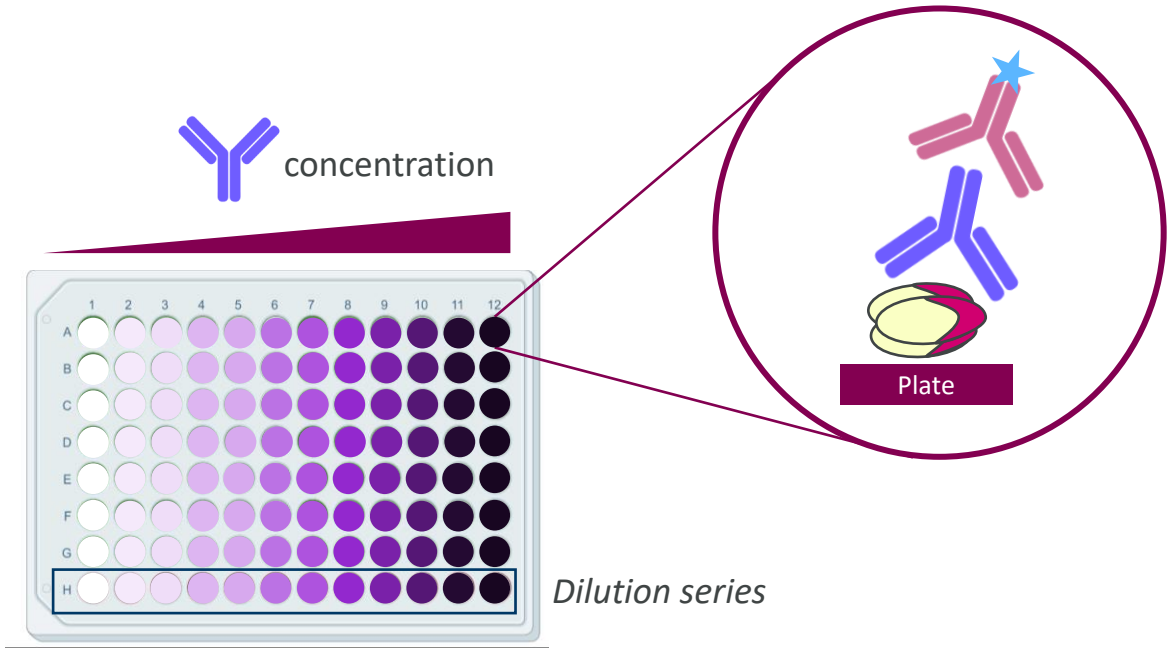

- Quantification of target binding

## Cell-based reporter assays

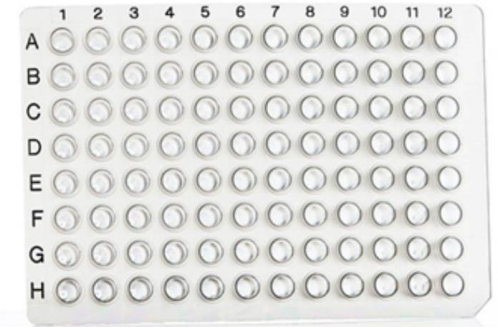


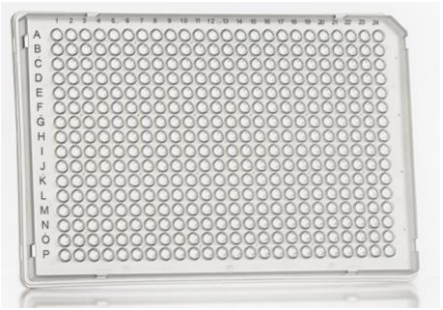

- Quantification of effect of target binding – activation/inhibition

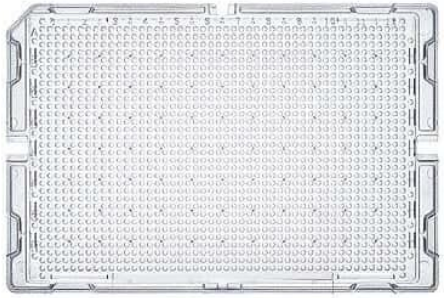
# Microtiter plates in potency assays



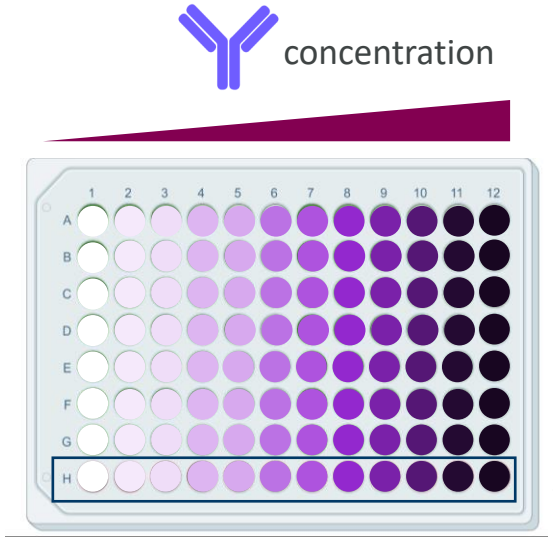

- Most common type: 96-well
- Less common: 384-well and 1536-well
- 1 assay run = one or multiple plates (depends on in-plate and plate-to-plate variability)
- On each plate – dilution series of a reference standard and samples



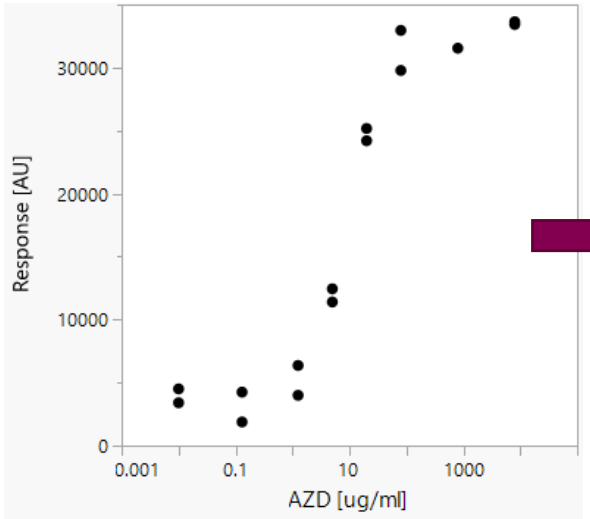


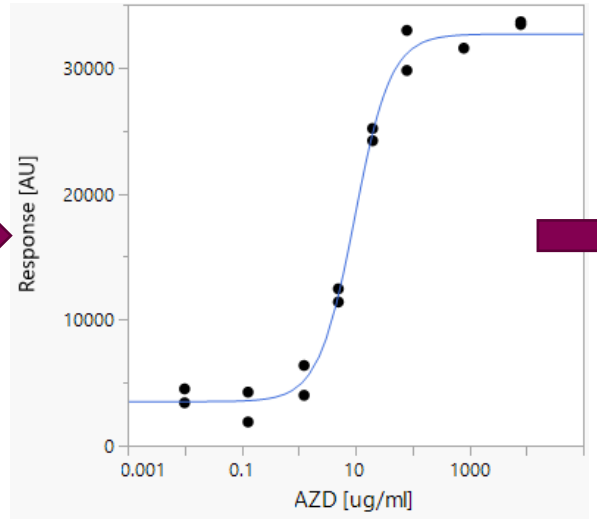
# Data and curve fitting in potency testing



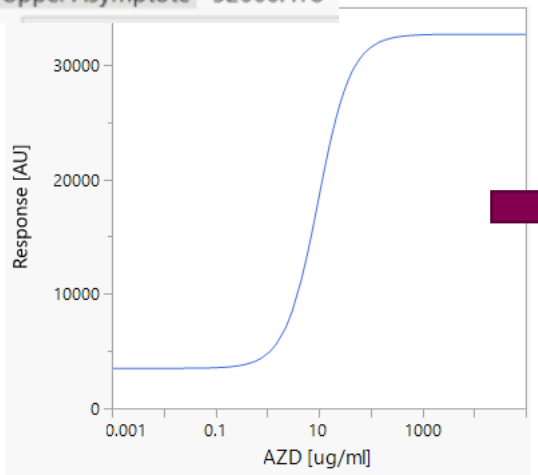

*Dilution series*

- Response data is used to generate a dose-response model fit (non-linear – 4PL or based on data transformation, e.g., log10)
- Pairwise comparison of fits between the standard and sample with shared lower and upper asymptotes, and growth rate
- Inflection point ($EC_{50}$ or $IC_{50}$) difference = % relative potency

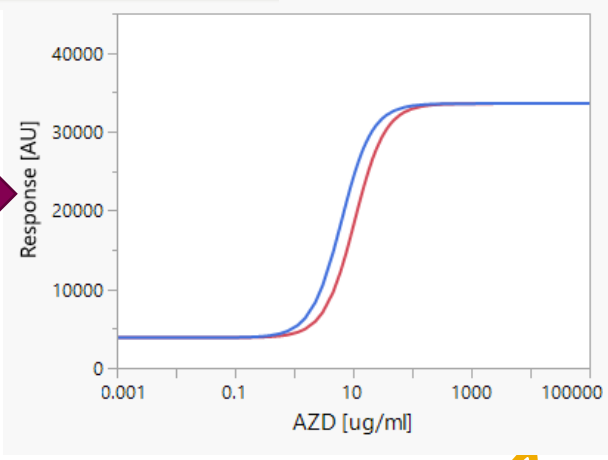


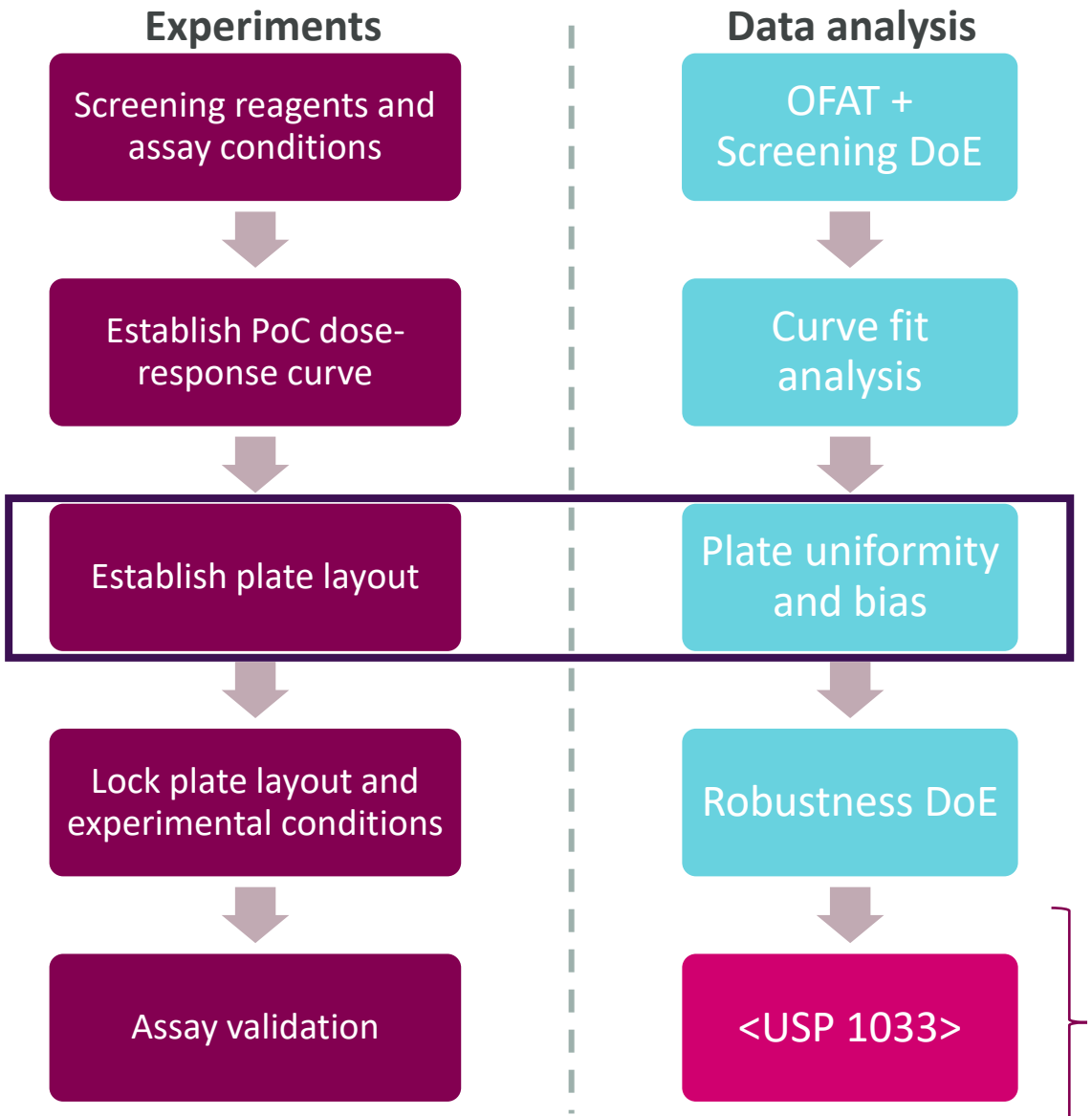

| Parameter | Estimate |
|---|---|
| Growth Rate | -1.369586 |
| Inflection Point | 9.6884903 |
| Lower Asymptote | 3492.698 |
| Upper Asymptote | 32666.478 |




| Parameter | Group |
|---|---|
| Growth Rate | |
| Lower Asymptote | |
| Upper Asymptote | |
| Inflection Point | RS |
| Inflection Point | S1 |



# Potency assay development



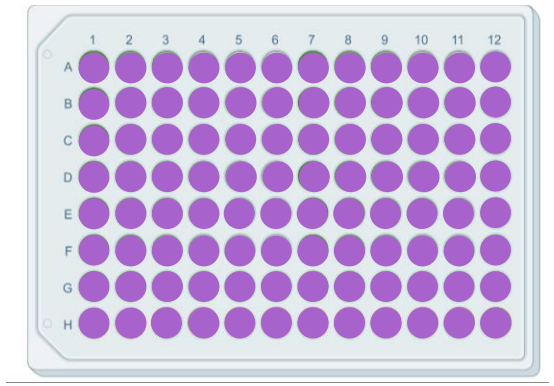

Can be performed with JMP

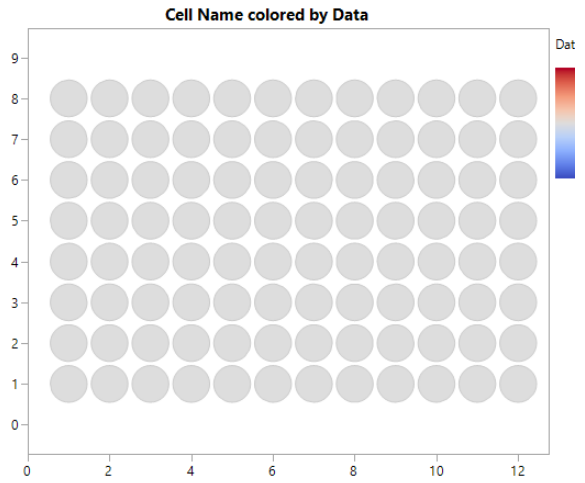
- Accuracy
- Linearity
- Precision
- Range
- Specificity
- Stability - indicating potential

# Plate bias

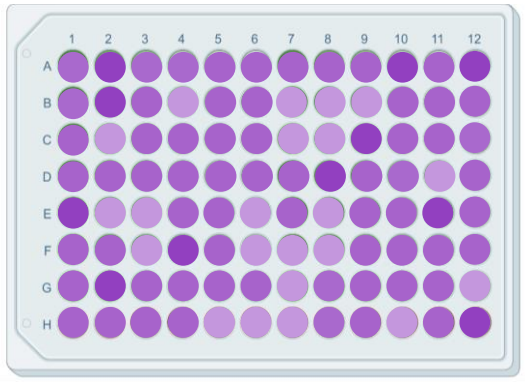
Constant concentration

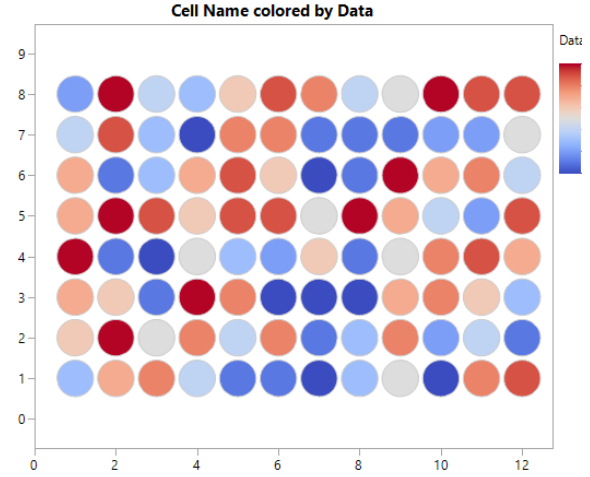
Ideally…



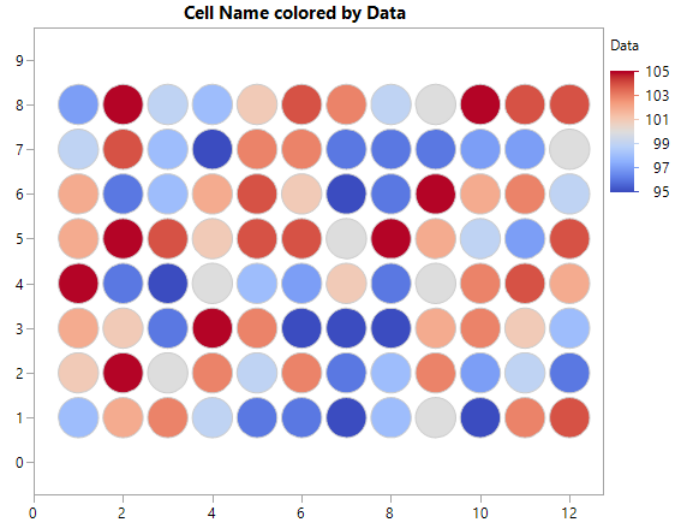

In reality…

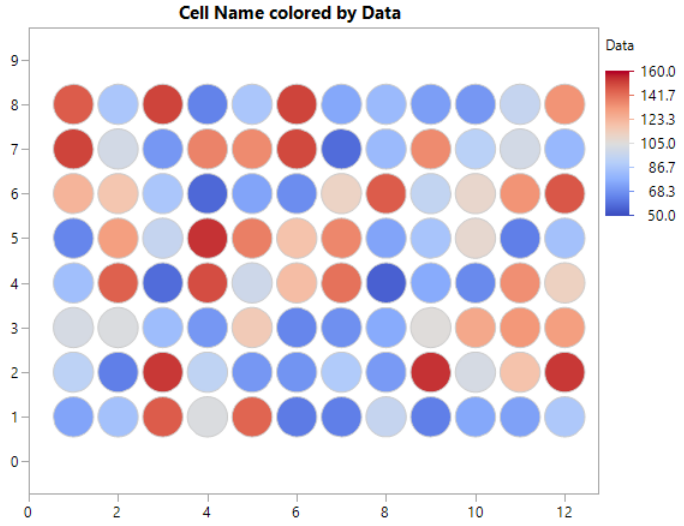


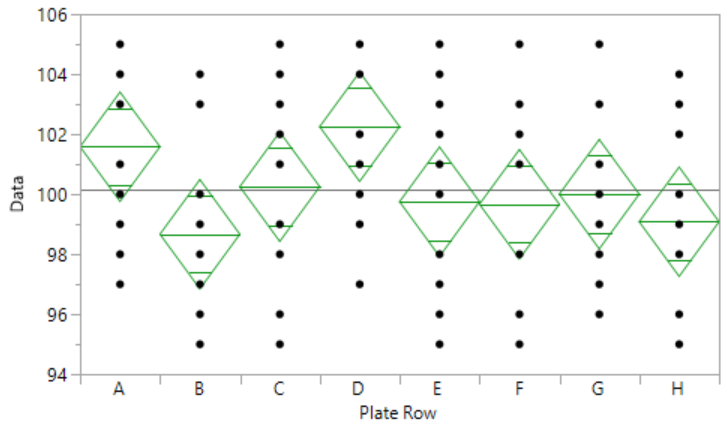

Variability is inevitable but needs to be understood and controlled…

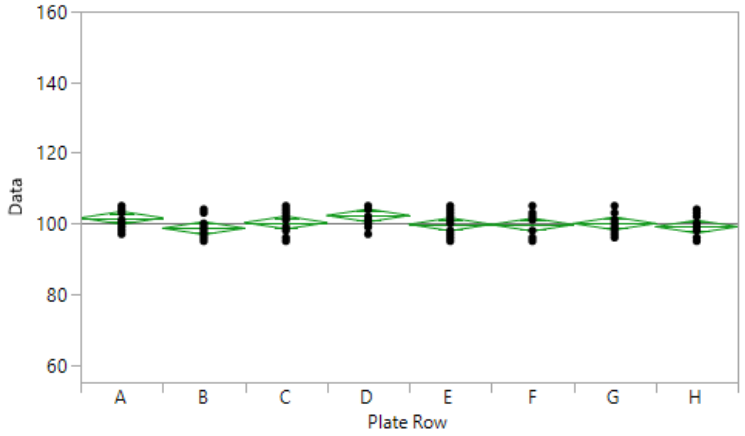
# Scale matters!

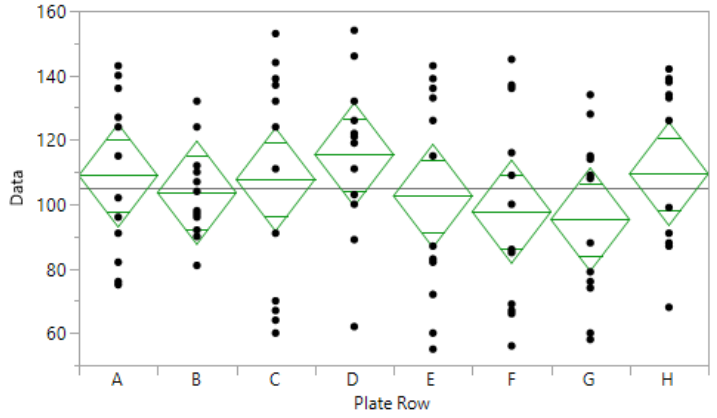


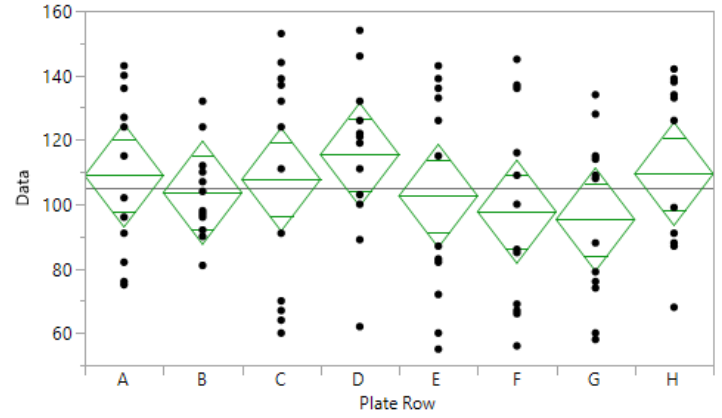




Analysis by plate row



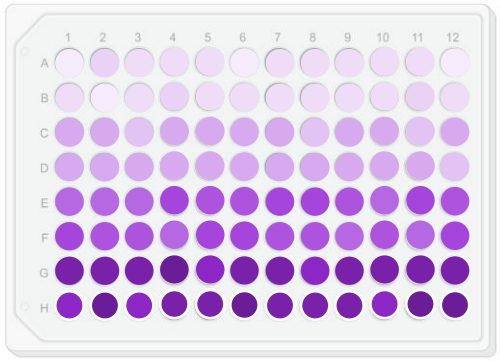



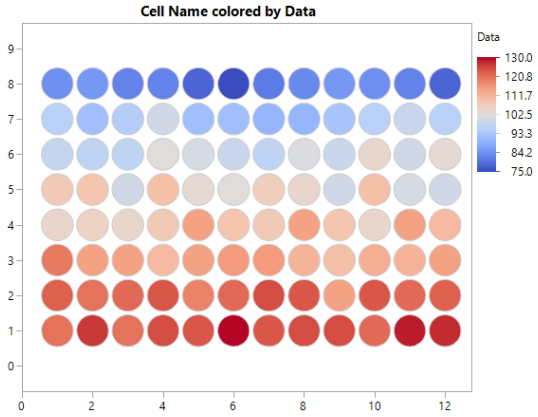

JMP offers great graphical and statistical tools for plate bias analysis

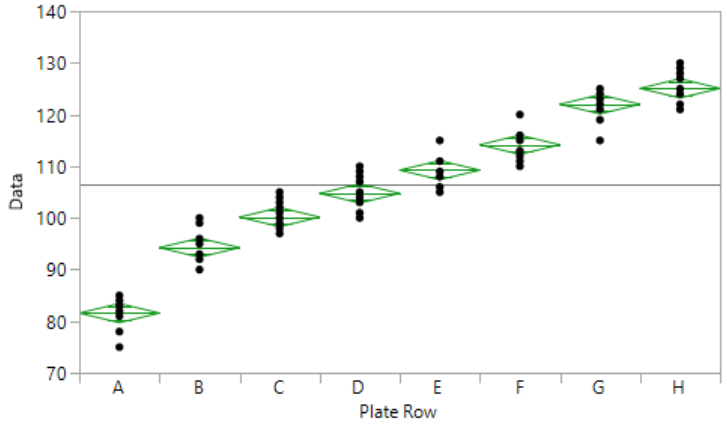
# Patterns of variability can be non-random

Constant concentration

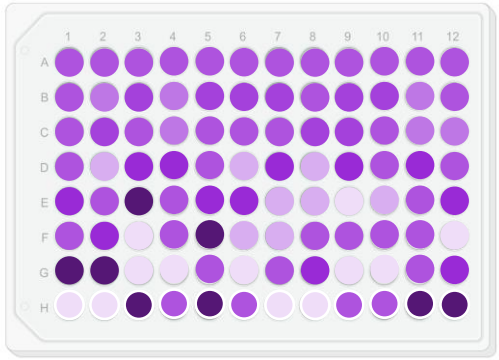



## Changes in average responses

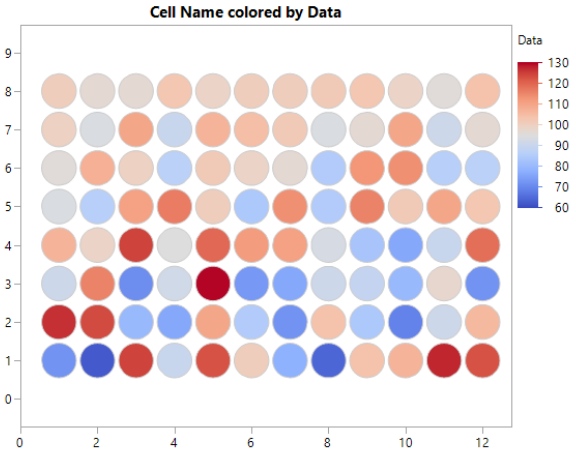


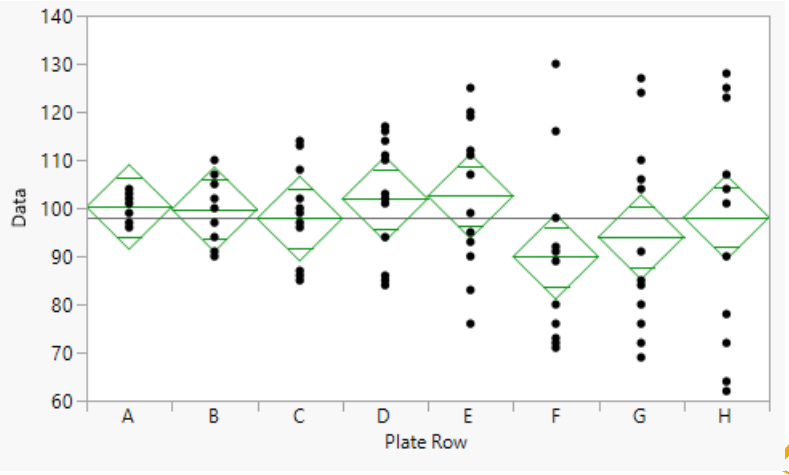




Constant concentration

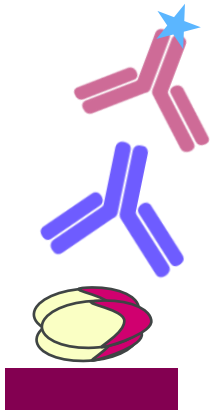



## Changes in variability



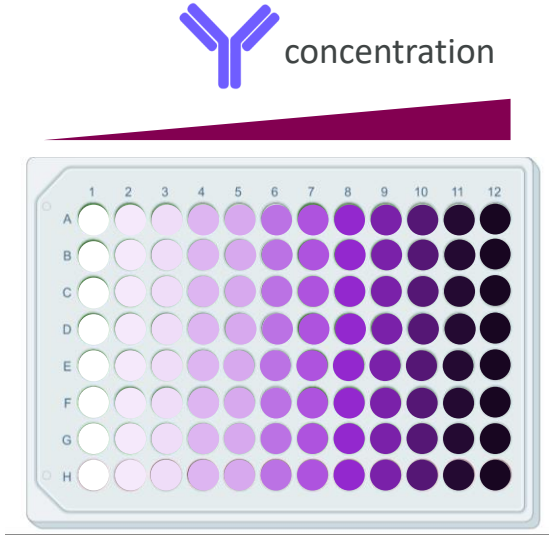
# Case study – plate uniformity

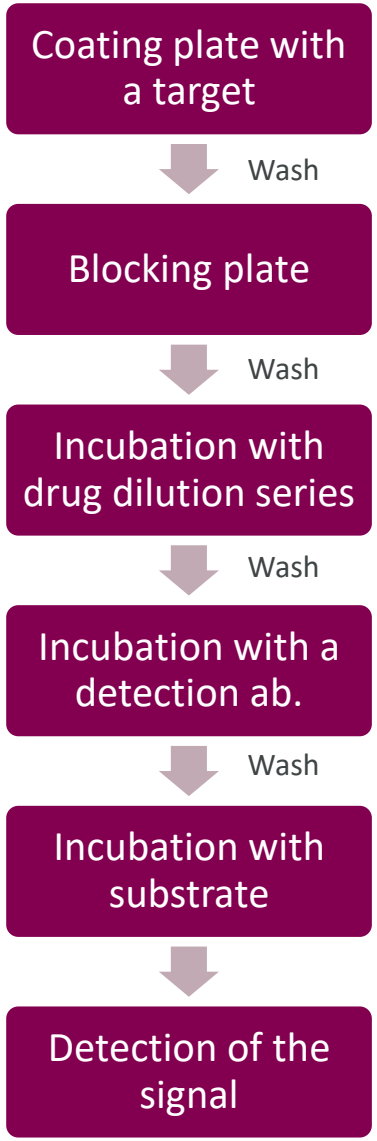


*Anti-human ab. (detection reagent)*

*AZ drug: human ab. (dilution series)*

*Recombinant target coated on a microtiter plate*




- Many binding assays have similar step but they can differ + differences in liquid volumes, incubation times, buffers, reagent concentrations and plate types used
- Plate uniformity has to be conducted for every assay we develop
- Many potential sources of variability, including the operator

# Old analysis workflow

Manual data import into Excel sheet

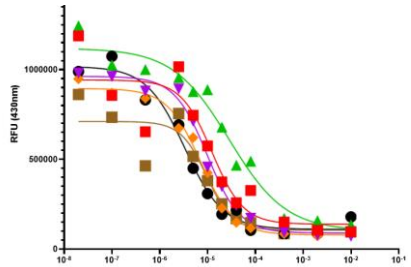
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 147298 | 95063 | 155113 | 248932 | 381189 | 472571 | 622195 | 792610 | 789928 | 911413 | 974151 | 966787 |
| B | 111261 | 115103 | 164243 | 289525 | 382309 | 529303 | 644538 | 772880 | 824252 | 960375 | 900931 | 1038538 |
| C | 84199 | 118285 | 184377 | 283457 | 391012 | 527619 | 632343 | 739465 | 795887 | 862319 | 942187 | 912590 |
| D | 78933 | 105116 | 157192 | 267545 | 360035 | 501838 | 568592 | 661869 | 804187 | 868023 | 833464 | 935807 |
| E | 89723 | 111844 | 170371 | 279681 | 356420 | 484348 | 607856 | 665541 | 720172 | 896118 | 842246 | 912021 |
| F | 78340 | 441289 | 215782 | 309114 | 373825 | 519291 | 586907 | 634168 | 757153 | 843640 | 813986 | 845250 |
| G | 119243 | 111428 | 177396 | 341834 | 355983 | 517056 | 548173 | 681025 | 780678 | 824763 | 812720 | 875179 |
| H | 89354 | 101020 | 166293 | 262272 | 328736 | 464464 | 547010 | 659087 | 678195 | 809315 | 773008 | 876827 |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 121711 | 133005 | 196830 | 327876 | 457132 | 641027 | 859453 | 938736 | 1009290 | 1241743 | 1208052 | 1279647 |
| B | 116383 | 136602 | 224170 | 378906 | 510420 | 692100 | 869513 | 951167 | 1137608 | 1240392 | 1176298 | 1339456 |
| C | 99774 | 131209 | 210888 | 347190 | 457113 | 586257 | 707171 | 783594 | 862590 | 957310 | 1015794 | 1004954 |
| D | 98273 | 117370 | 177106 | 308017 | 421774 | 545111 | 622105 | 746883 | 773628 | 920647 | 856103 | 936994 |
| E | 91794 | 130316 | 176216 | 288193 | 390588 | 510718 | 596659 | 726180 | 774492 | 836622 | 884994 | 886163 |
| F | 135657 | 147475 | 196836 | 312163 | 420634 | 573277 | 614320 | 669663 | 760327 | 864342 | 789661 | 904833 |
| G | 103496 | 141811 | 280576 | 328260 | 378631 | 498058 | 534338 | 615147 | 727544 | 809250 | 849452 | 865072 |
| H | 99025 | 131288 | 207749 | 284266 | 375486 | 698471 | 606138 | 611243 | 1559314 | 832107 | 778320 | 863049 |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 226127 | 118639 | 162234 | 255913 | 366232 | 484506 | 579192 | 823285 | 748084 | 854353 | 910970 | 920259 |

Manual data arrangement into dilution series/plate

Mean RFU

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Plate 1 | 99793.875 | 149893.5 | 173845.9 | 285295 | 366188.6 | 502061.3 | 594701.8 | 70 |
| Plate 2 | 108264.125 | 133634.5 | 208796.4 | 321858.9 | 426472.3 | 593127.4 | 676212.1 | 75 |
| Plate 3 | 148931.5 | 162469.125 | 181216.8 | 276226.9 | 353531.1 | 443446.4 | 508105.8 | 60 |
| Plate 4 | 81892.75 | 105314.25 | 153469.5 | 260233.1 | 360926.1 | 471483.1 | 572264 | 66 |
| Plate 5 | 107281.875 | 125998.75 | 188056.1 | 299591.8 | 402044 | 546349.5 | 684767.6 | 85 |
| Plate 6 | 95177.5 | 112783.875 | 172223.1 | 254889.5 | 358363 | 465619.6 | 543903.8 | 63 |

$EC_{50}$/$IC_{50}$ calculation in a different software




| | | | |
|---|---|---|---|
| IC50 | 1.241e-005 | 2.839e-005 | 9.804e-006 |

Manual inflection point import into Excel

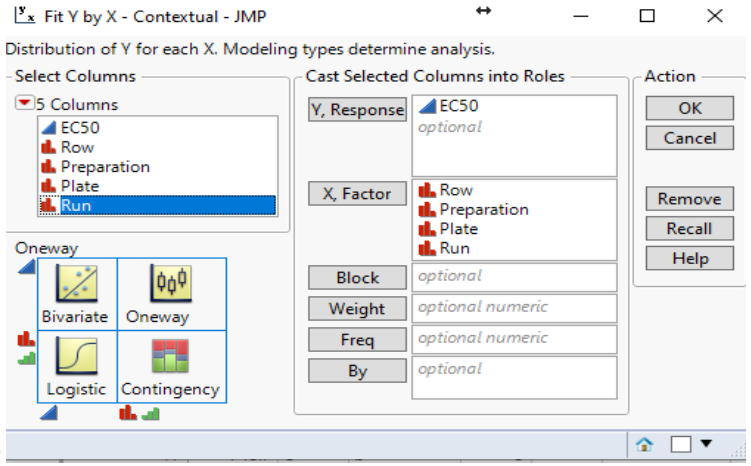
| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| Plate 1 | 44.94 | 50.45 | 59.97 | 52.21 | 50.89 | 38.98 | 61.34 | 52.21 |
| Plate 2 | 46.57 | 54.64 | 65.81 | 67.01 | 60.76 | 71.29 | 49.63 | 68.22 |
| Plate 3 | 45.48 | 68.02 | 85.78 | 32.41 | 62.38 | 46.06 | 36.64 | 52.52 |
| Plate 4 | 41.63 | 43.05 | 55.89 | 60.85 | 53.39 | 56.7 | 56.18 | 53.47 |
| Plate 5 | 38.88 | 48.76 | 68.42 | 49.46 | 64.56 | 64.93 | 53.07 | 44.93 |
| Plate 6 | 40.74 | 46.65 | 54.03 | 57.98 | 62.19 | 54.02 | 41.69 | 34.11 |

Manual data arrangement in Excel

JMP Table

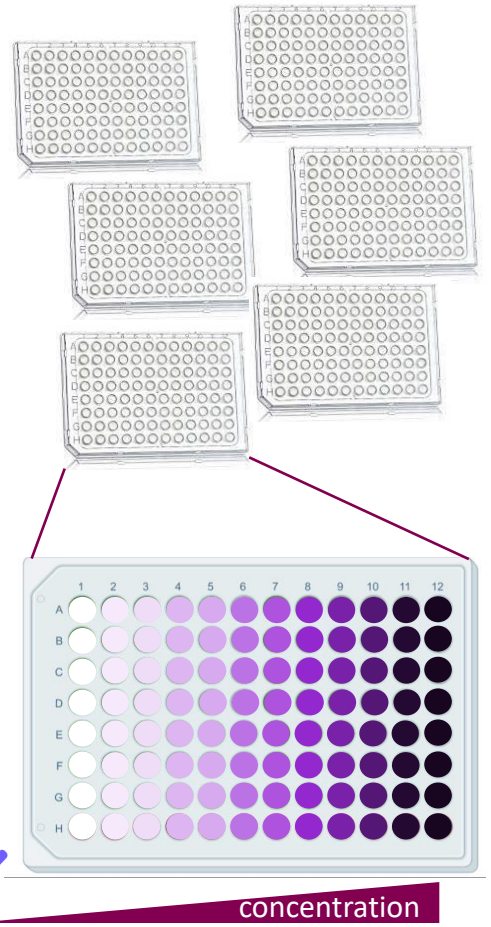
| Plate | Row | IC50 | Prep | Run |
|---|---|---|---|---|
| 1 | A | 44.94 | 1 | 1 |
| 1 | B | 50.45 | 1 | 1 |
| 1 | C | 59.97 | 1 | 1 |
| 1 | D | 52.21 | 1 | 1 |
| 1 | E | 50.89 | 1 | 1 |
| 1 | F | 38.98 | 1 | 1 |
| 1 | G | 61.34 | 1 | 1 |
| 1 | H | 52.21 | 1 | 1 |
| 2 | A | 46.57 | 2 | 1 |

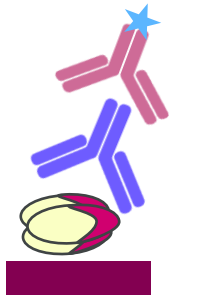
Manual analysis in JMP

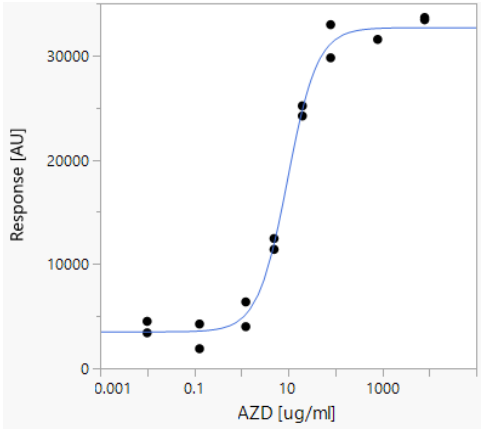


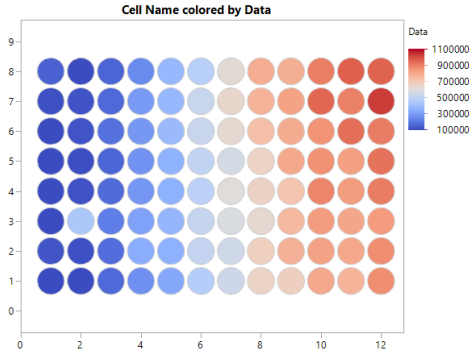
# Improved analysis workflow

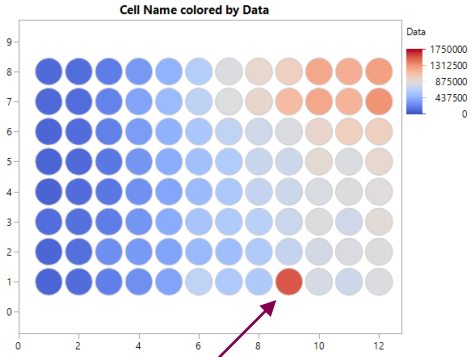
Data import into JMP → Plate heatmaps generation → Curve bias analysis



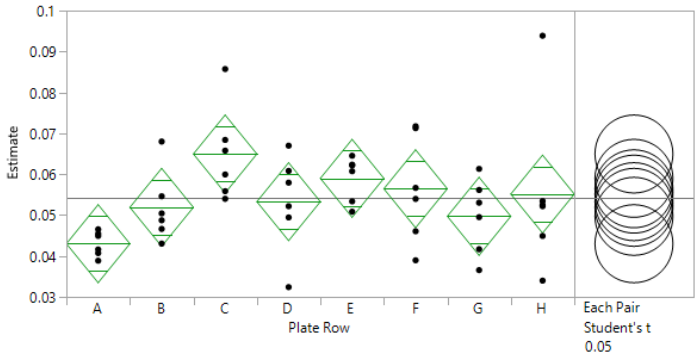





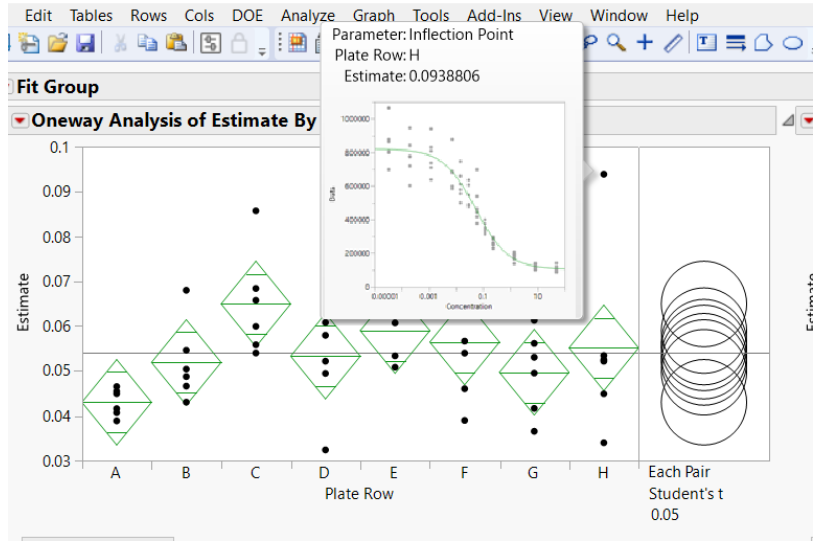




Detection and elimination of outliers (“hot spots”)




Fully employing JMP interactiveness



# Demonstration in JMP

# Benefits of the new tool

- Removal of manual, error-prone copy-paste of data
- “One stop-shop” for plate bias and uniformity analysis with most statistically sound models already chosen for users
- Plate heat map generation options allow removal of obvious data outliers to focus on “true” variability analysis
- Interconnectivity of curve analysis with plate row/column variability analysis
- More in-depth analysis of uncertainty in calculation of $EC_{50}$/$IC_{50}$ possible

Aiding and speeding up development of robust potency assays

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