



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

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JMP Discovery Summit

March 2023



Acknowledgements

Biopharmaceutical Development, AstraZeneca:

- Deepika Srivastava
- Barbara Hebeis



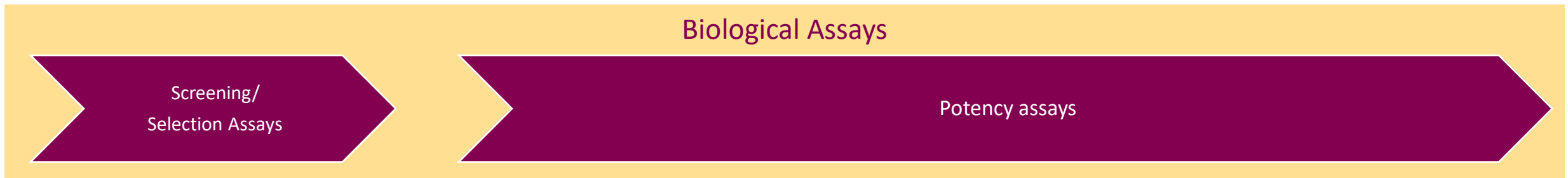


Presentation outline

- 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

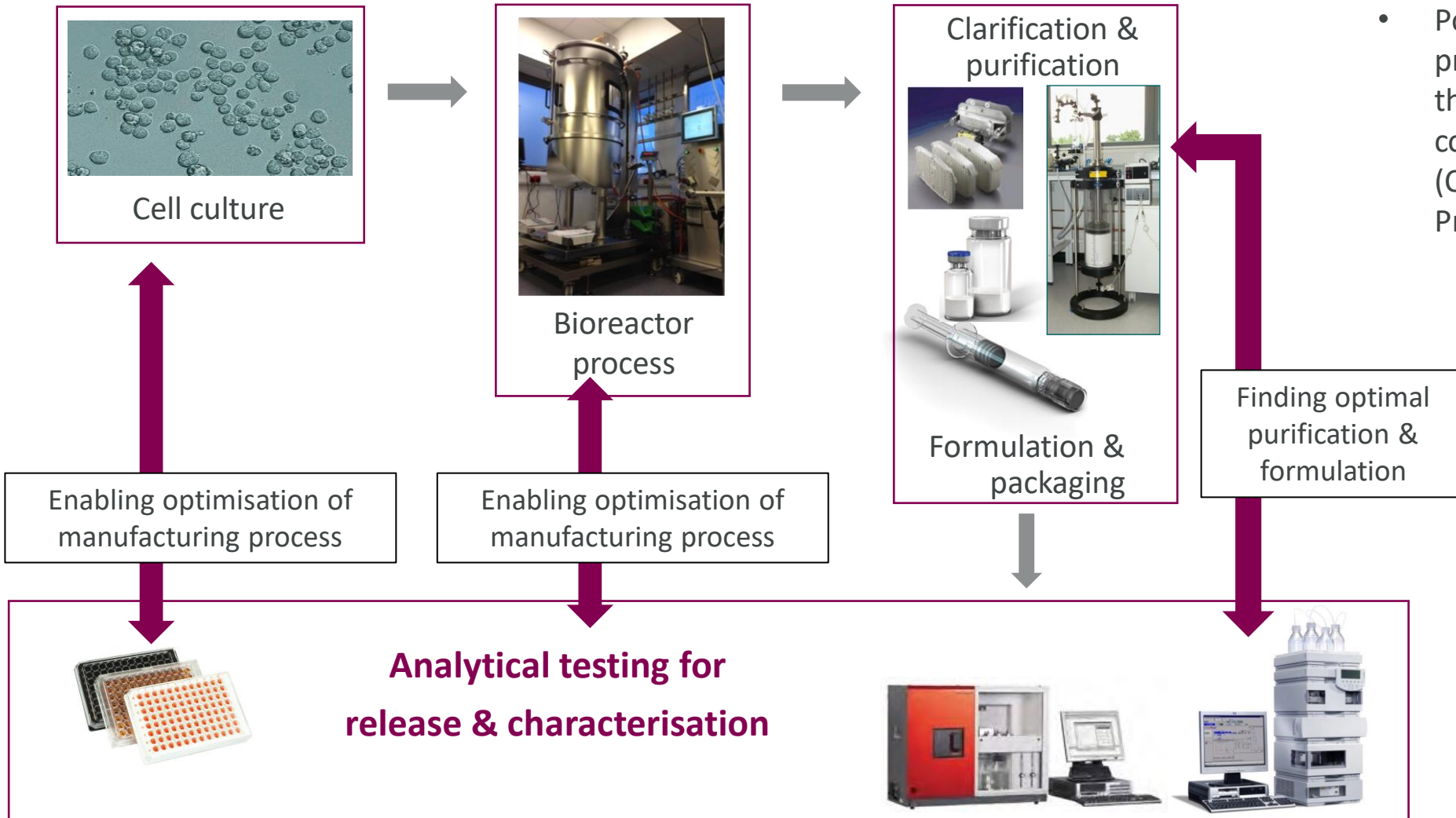


Which molecule has biological activity?

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



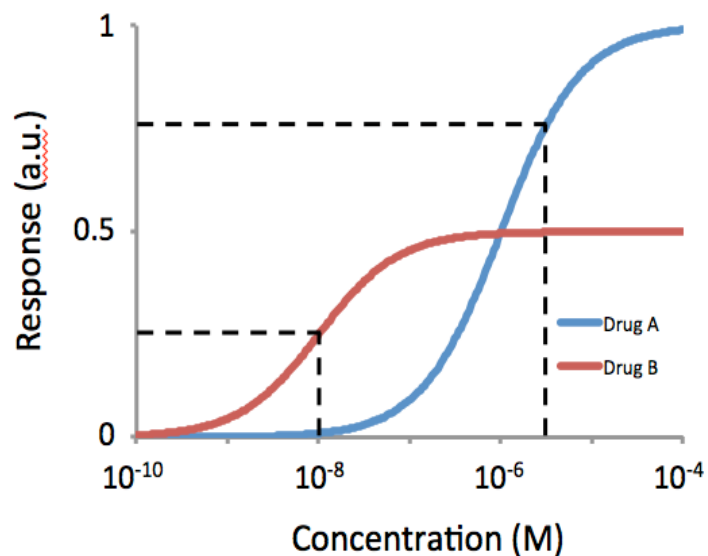
Potency assays as part of a typical biopharmaceutical manufacturing process



- 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)

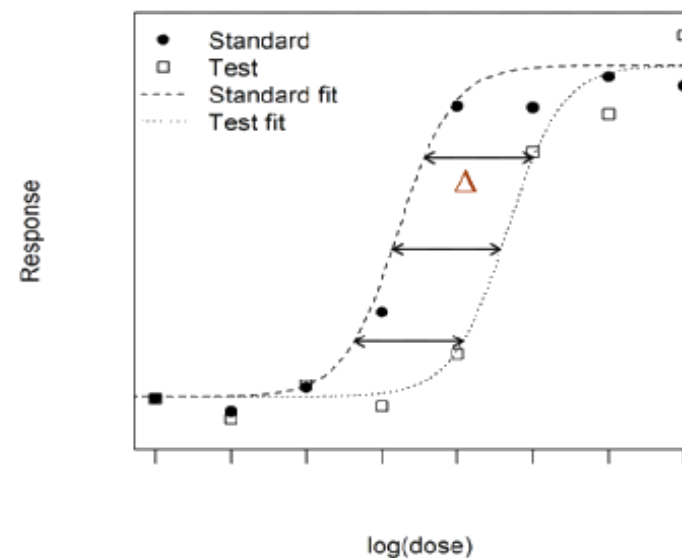


Screening vs potency assays - measuring biological activity



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



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

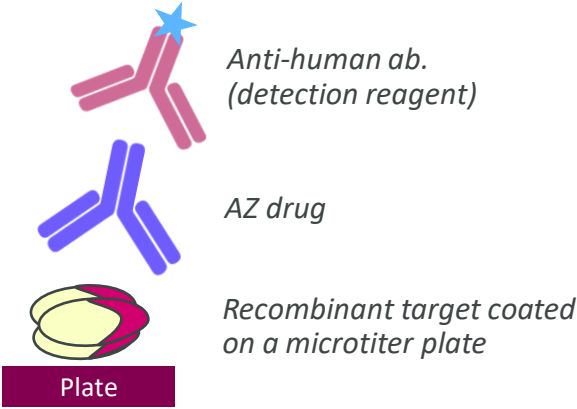
CMC Potency Assays:
Resolve within 2 fold change
between
different lots of the same drug



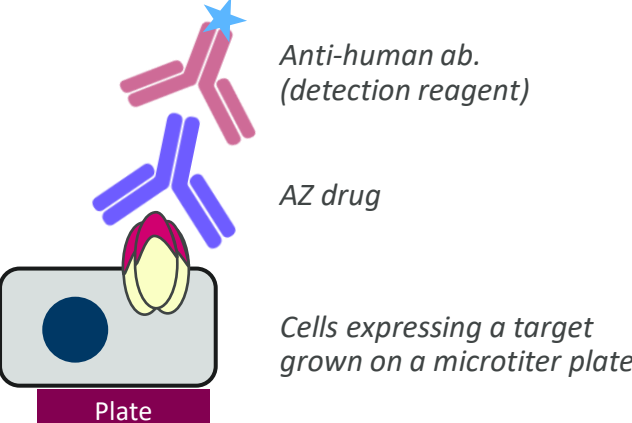
Common types of potency assays



Non cell-based target binding assays

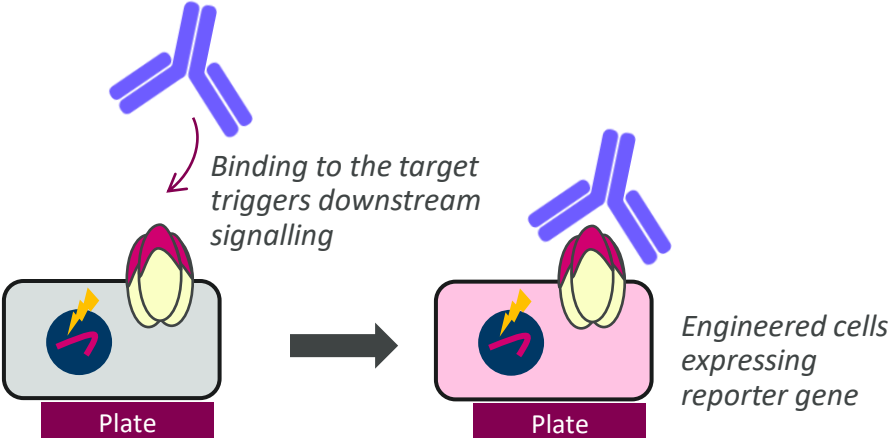


Cell-based target binding assays



- Quantification of target binding

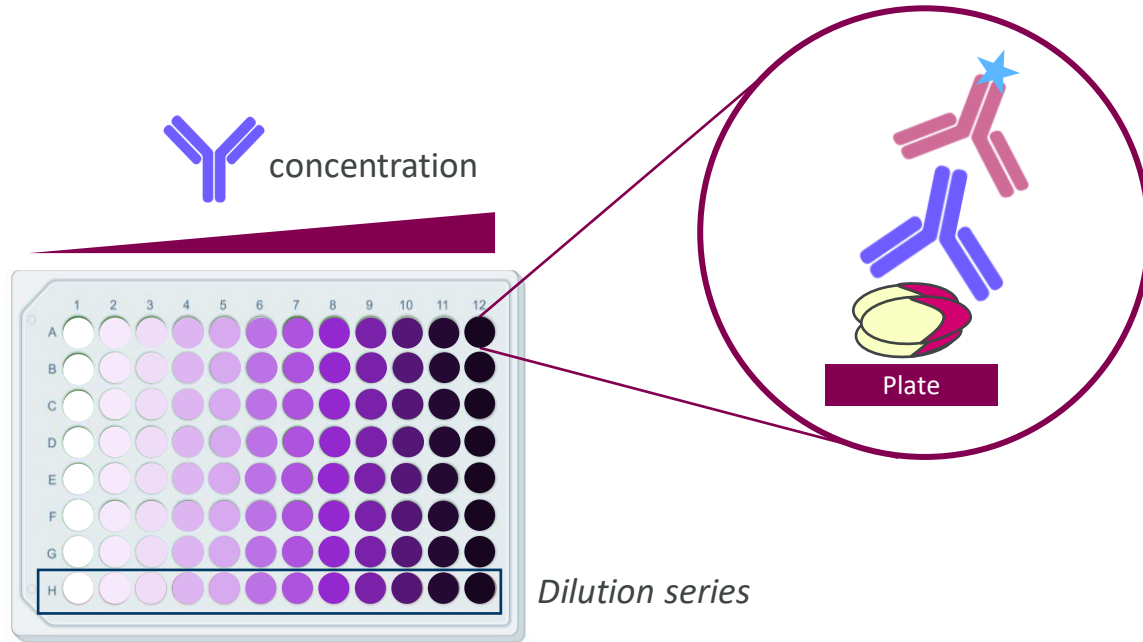
Cell-based reporter assays



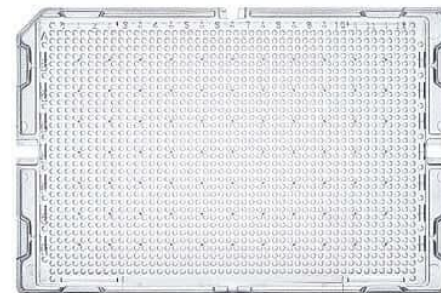
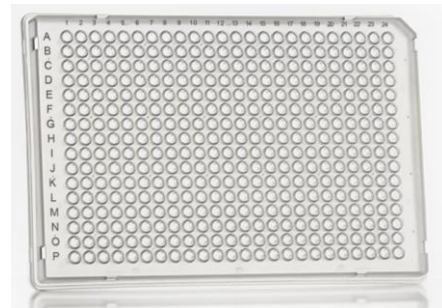
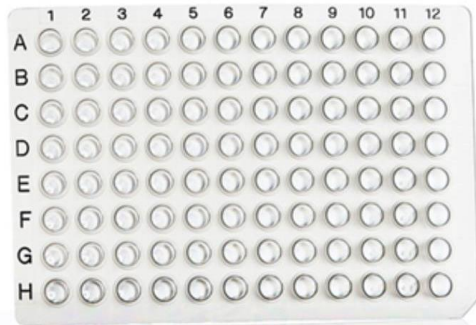
- Quantification of effect of target binding – activation/inhibition



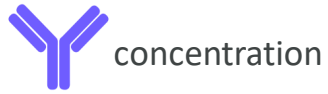
Microtiter plates in potency assays



- 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

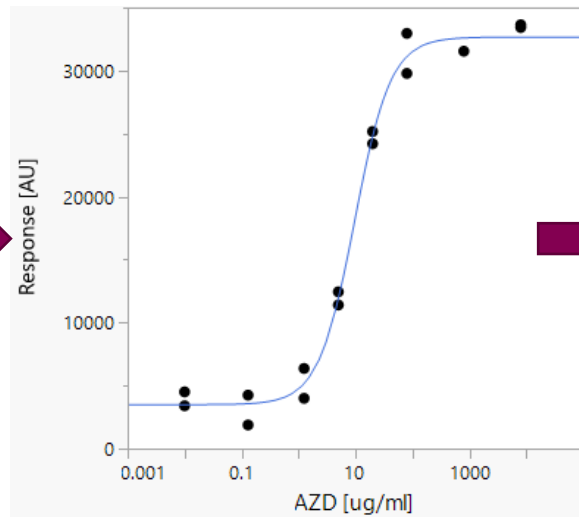
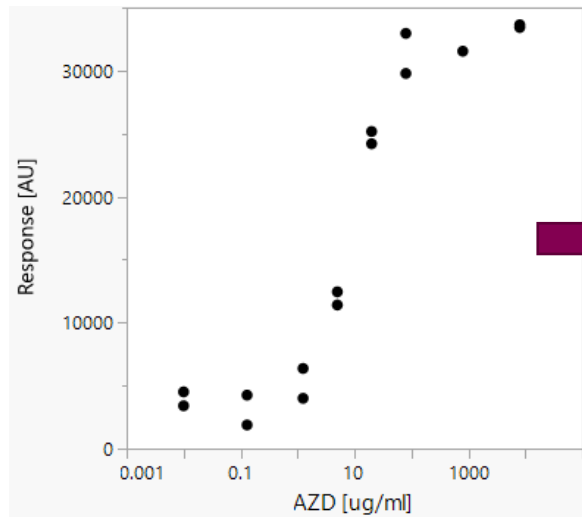


Data and curve fitting in potency testing

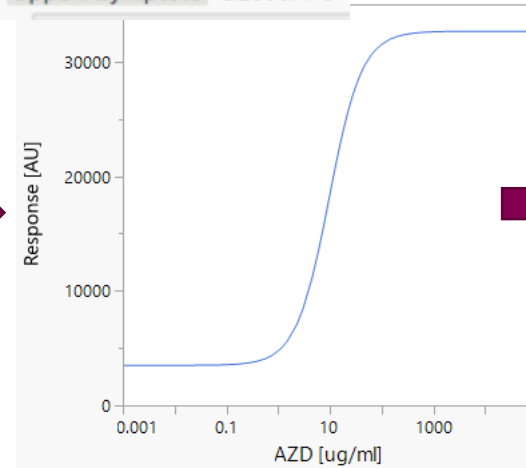


Dilution series

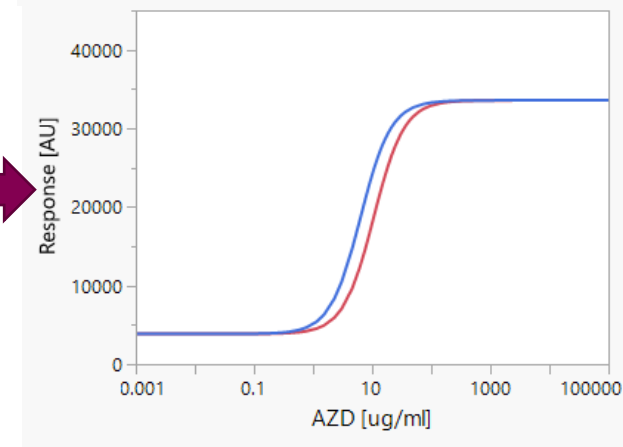
- Response data is used to generate a dose-response model fit (non-linear – 4PL or based on data transformation, e.g., log10)
- Pairwise comparison of fits between the standard and sample with shared lower and upper asymptotes, and growth rate
- **Inflection point** (EC_{50} or IC_{50}) difference =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

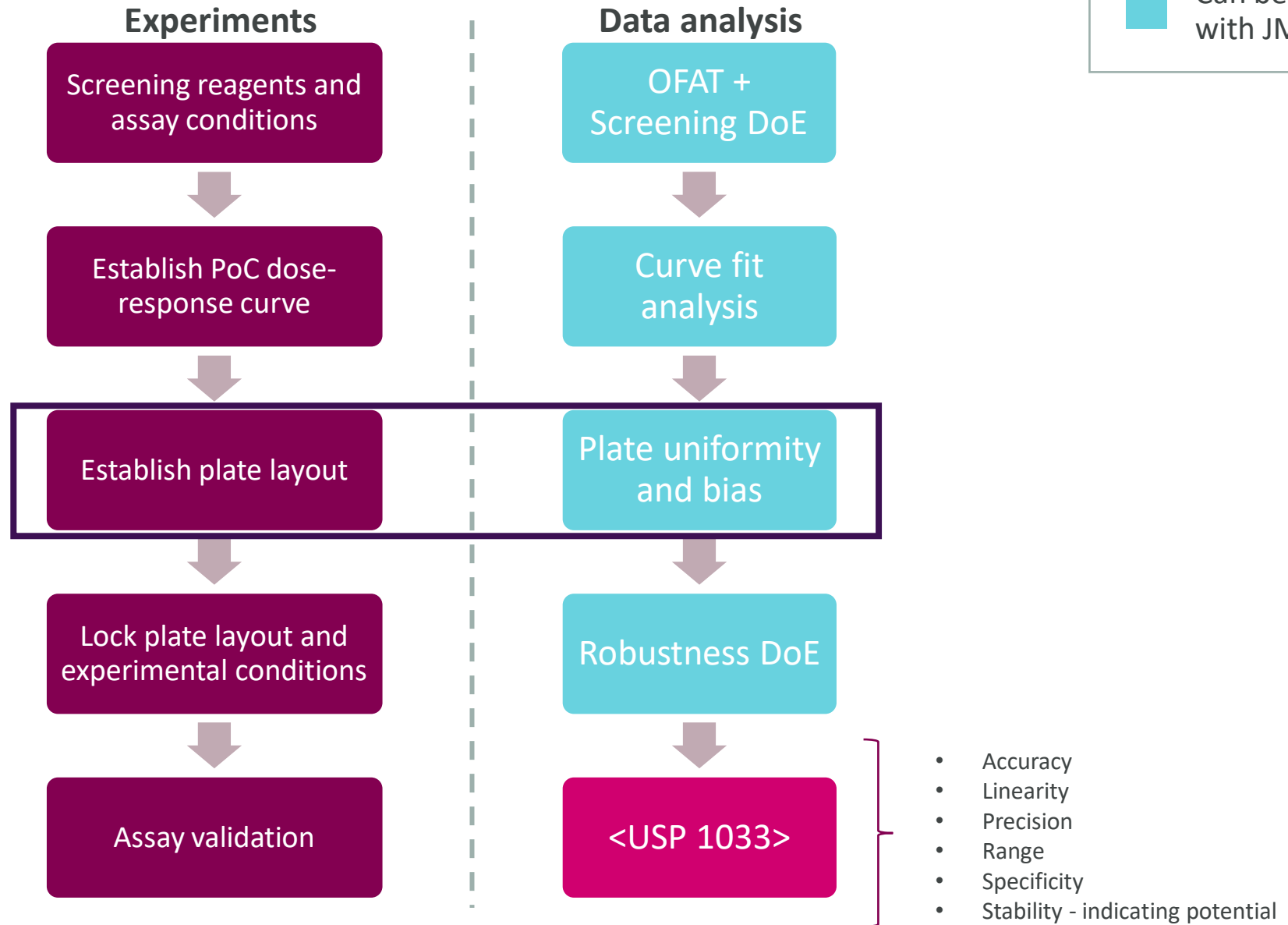
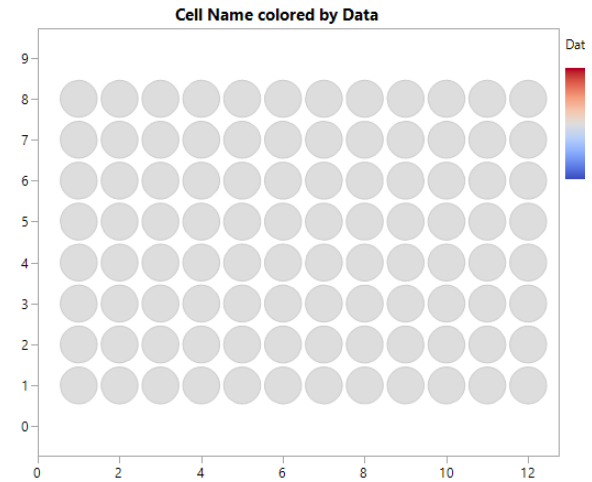
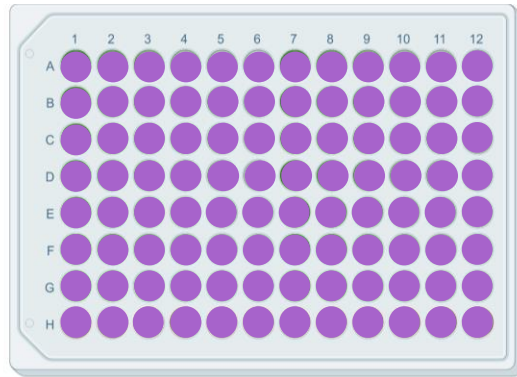


Plate bias



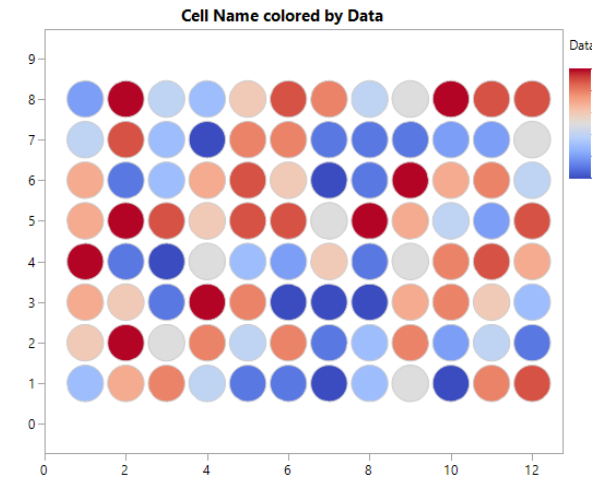
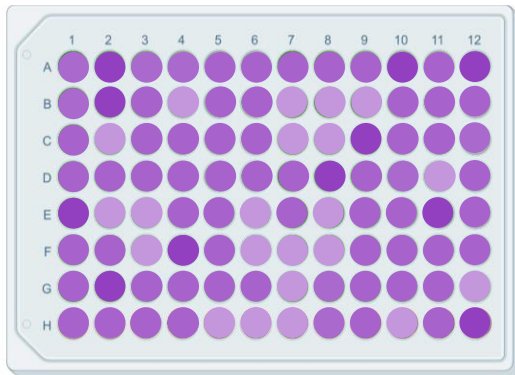
Constant concentration



Ideally...



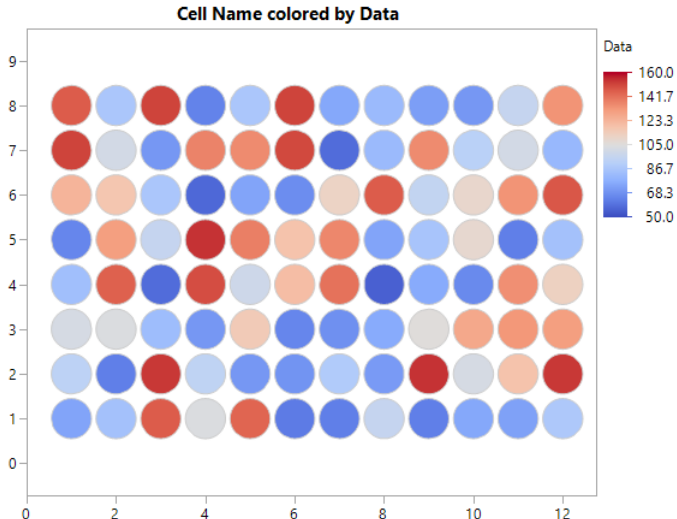
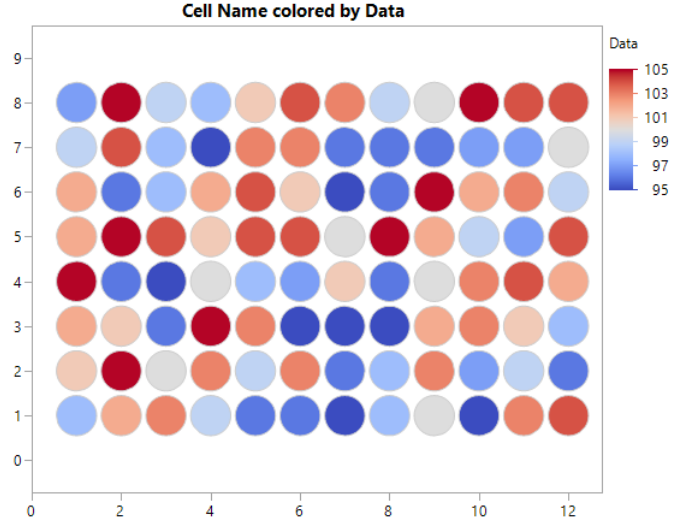
In reality...



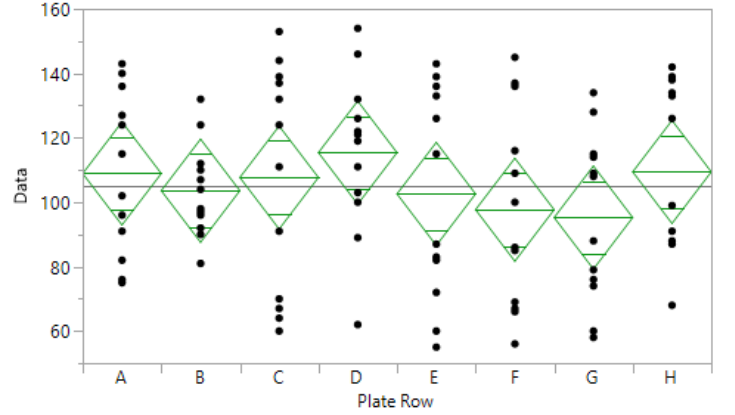
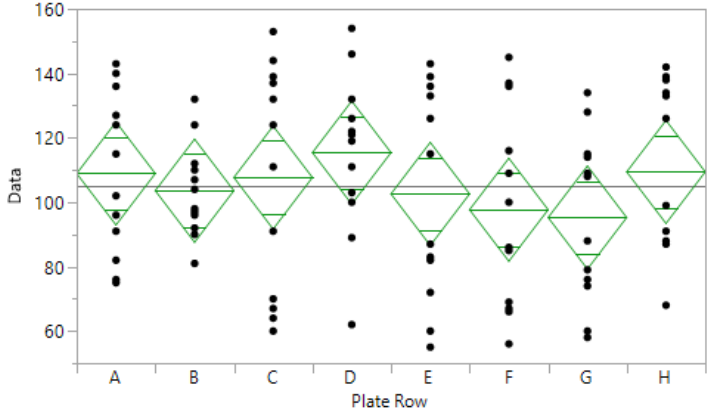
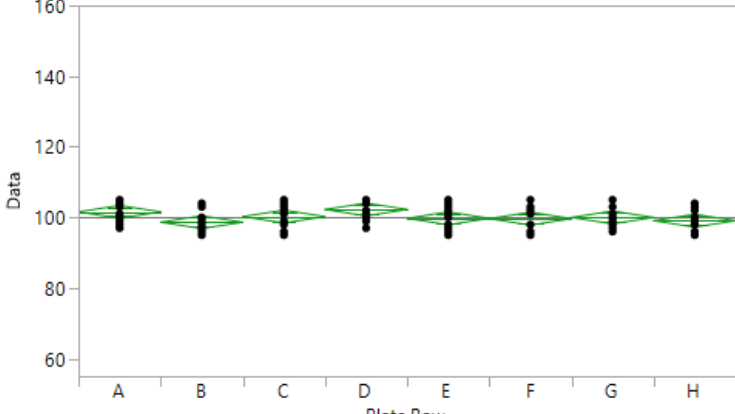
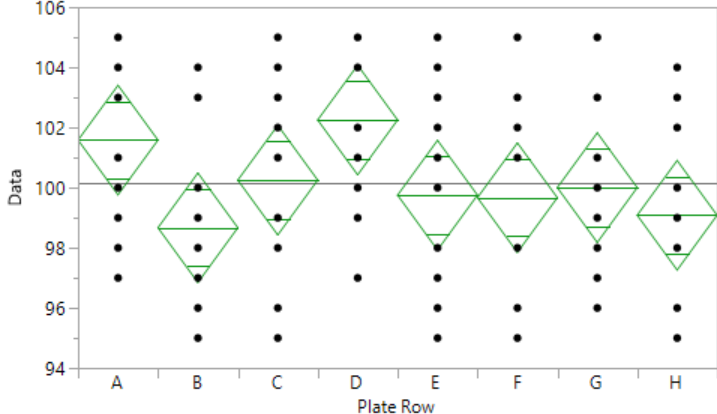
Variability is inevitable but needs to be understood and controlled...



Scale matters!



Analysis by plate row



JMP offers great graphical and statistical tools for plate bias analysis

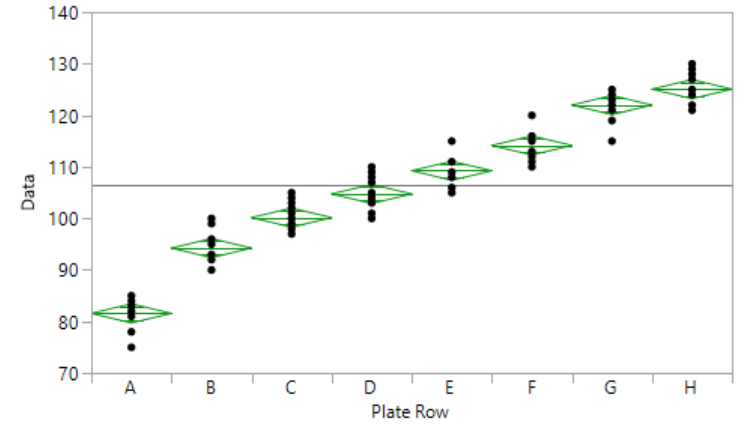
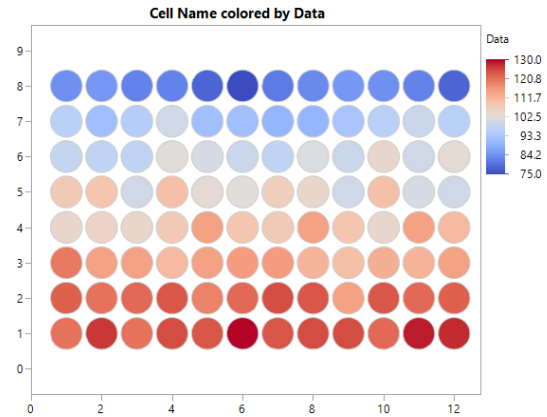
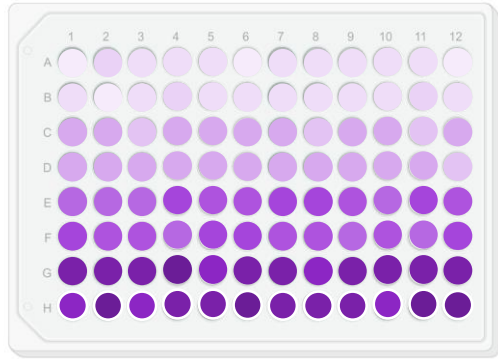


Patterns of variability can be non-random



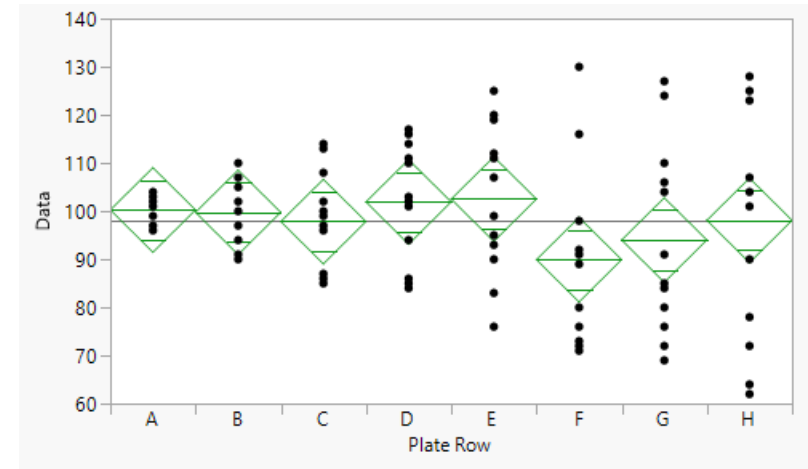
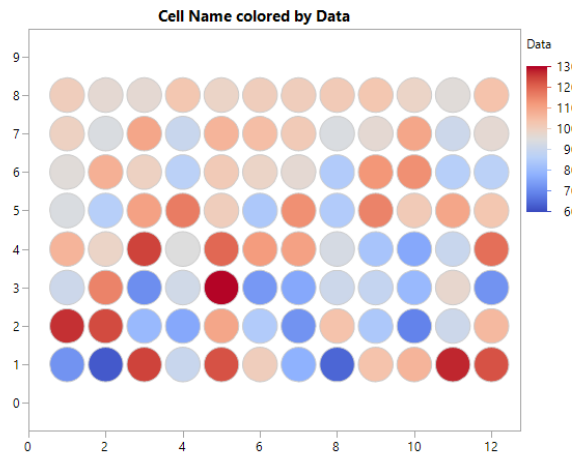
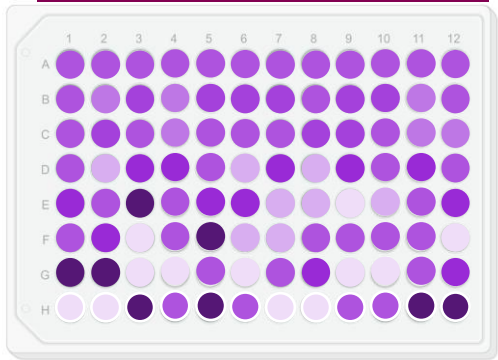
Constant concentration

Changes in average responses

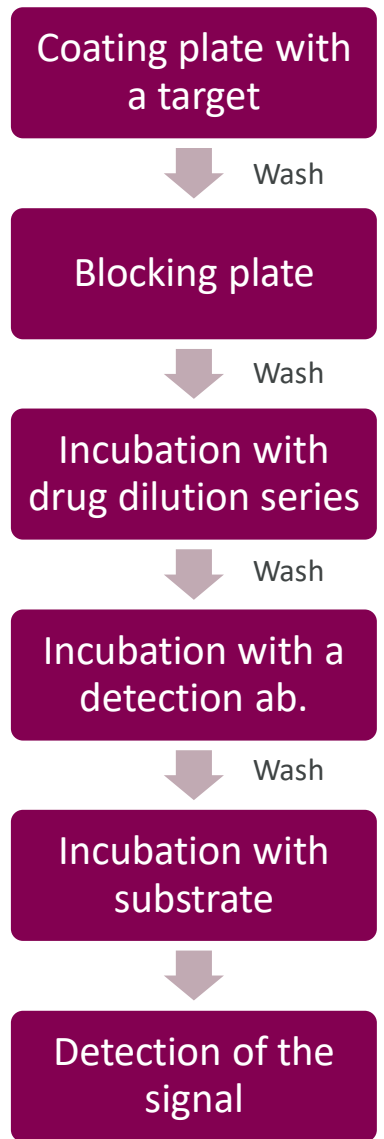
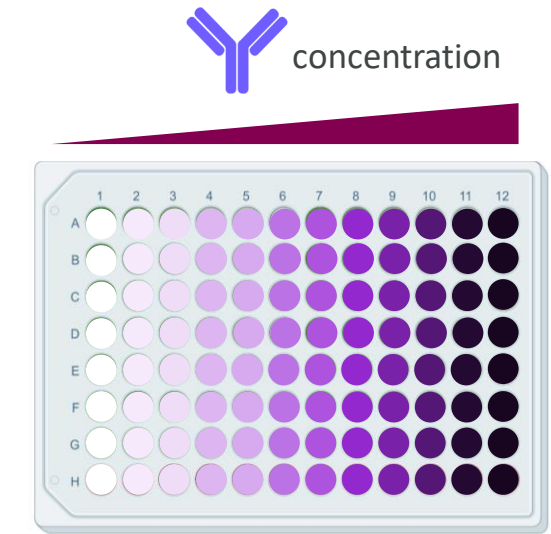
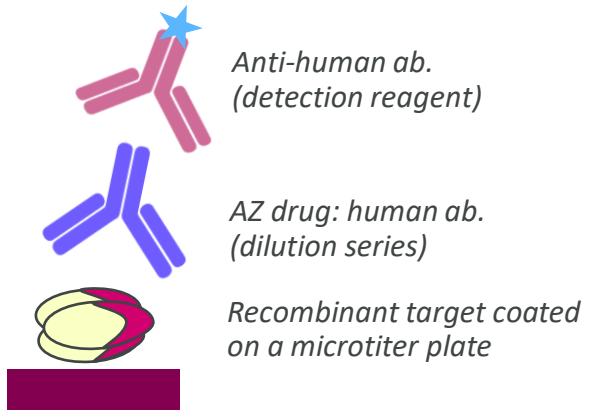


Constant concentration

Changes in variability



Case study – plate uniformity



- 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

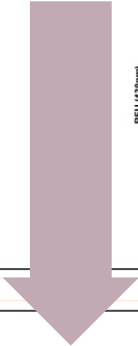
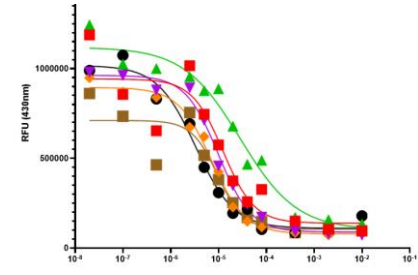
Manual data arrangement into dilution series/plate

EC₅₀/IC₅₀ calculation in a different software

	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



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				



Manual analysis in JMP

Manual data arrangement in Excel

Manual inflection point import into Excel



Fit Y by X - Contextual - JMP

Distribution of Y for each X. Modeling types determine analysis.

Select Columns: EC50, Row, Preparation, Plate, Run

Cast Selected Columns into Roles: Y, Response: EC50; X, Factor: Row, Preparation, Plate, Run

Block: optional; Weight: optional numeric; Freq: optional numeric; By: optional

One-way: Bivariate, Oneway, Logistic, Contingency

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

	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



Improved analysis workflow

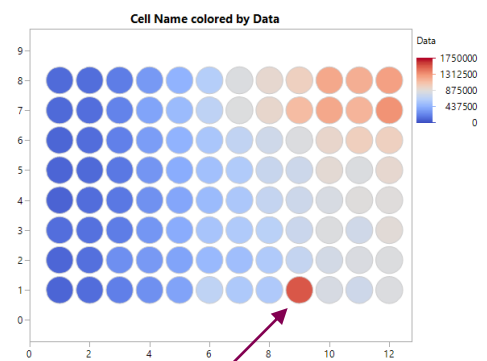
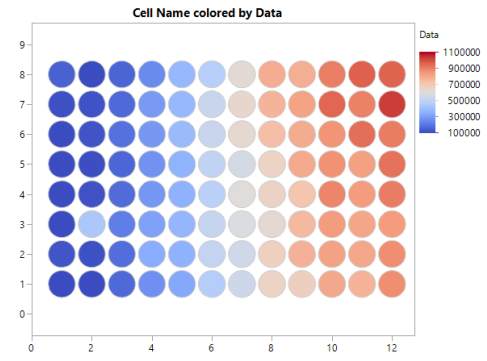
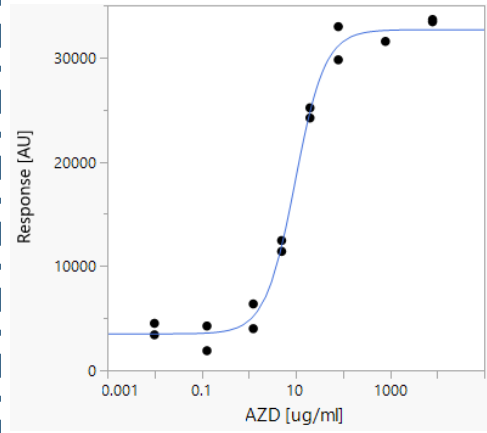
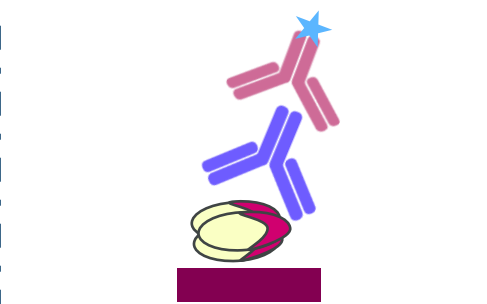
Data import into JMP



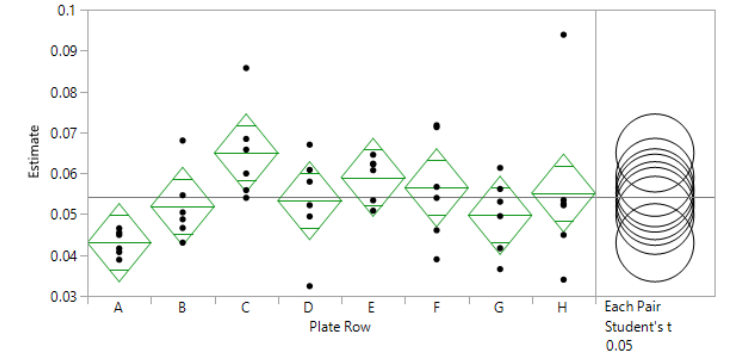
Plate heatmaps generation



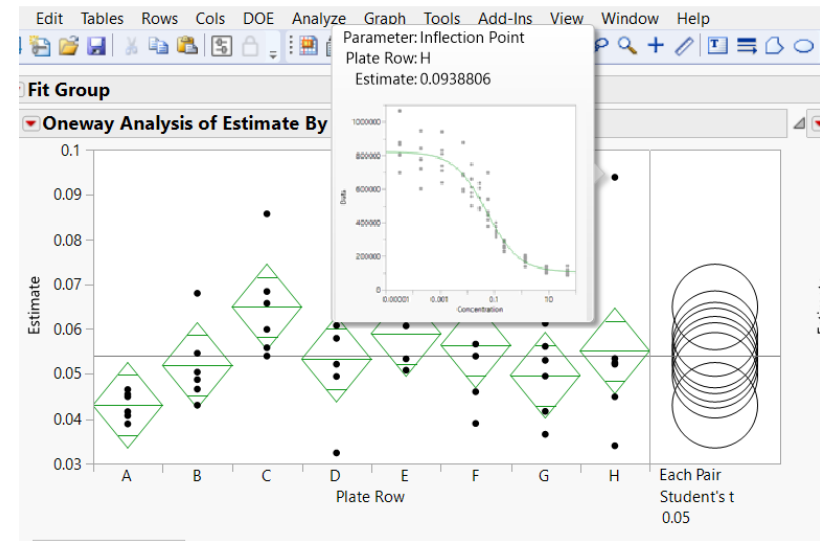
Curve bias analysis



Detection and elimination of outliers ("hot spots")



Fully employing JMP interactivensess



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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