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Setting up Acceptance Criteria with the Application of Equivalence Test and Geometric Mean Following USP Chapters - A Cell-based Potency Assay Case Study

PRESENTED BY:

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Pfizer ESSENTIAL HEALTH

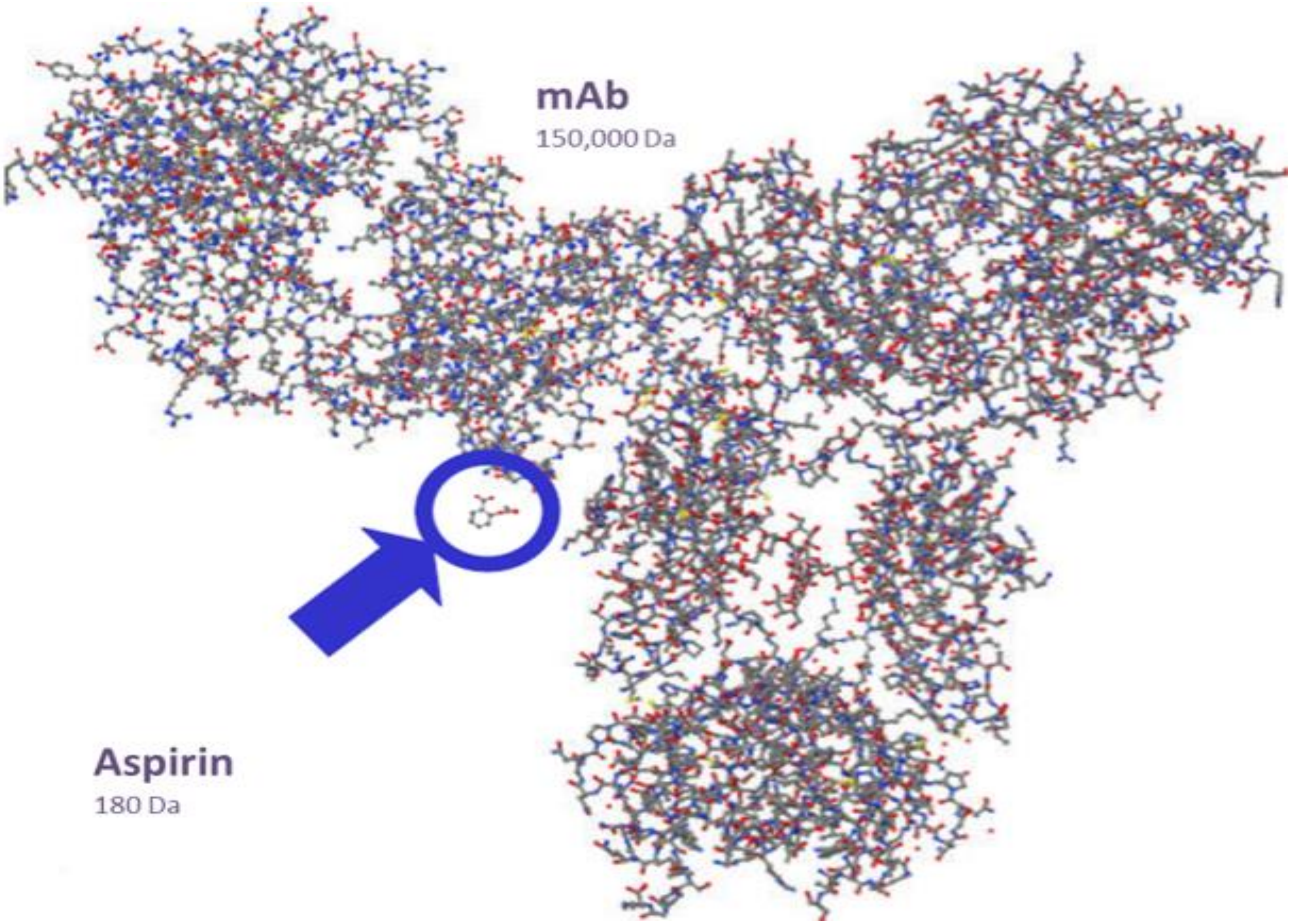
Outline

- Background information
- Assay DOE optimization
- Ready-to-use cell banks/method qualification
- Data driven and stage-appropriate acceptance criteria set up
- Control trending
- Summary and acknowledgement

USP recommendations

- Equivalence test has been recommended by USP for parallelism evaluation
  - Currently most popular *F*-test has obvious drawbacks
  - Assays with very good precision will fail parallelism test, but assays with poor precision may pass parallelism using *F*-test
- Geometric mean has been recommended by USP for final reportable result calculation
  - Majority of bioassay results are not normal distributed
  - Geometric mean will be better to address skewed results and be closer to true value

Biological products



Biological products (cont.)

Market Name	Drug Type	Mechanism of Action	Indication	Company	Sales in 2014
Humira	Mab	Anti-TNFα	Psoriasis, RA etc.	Abbvie	$12.54 b
Sovaldi	Small Molecule	HCV Polymerase Inhibitor	HCV Infection	Gilead	$10.28 b
Remicade	Chimeric Mab	Anti-TNFα	Psoriasis, RA etc.	Johnson & Johnson	$9.24 b
Rituxan	Chimeric Mab	Anti-CD20	Lymphoma, Leukemia etc.	Roche	$8.68 b
Enbrel	Fusion Protein	Bind TNFα	Psoriasis, RA etc.	Amgen	$8.54 b
Lantus	Insulin Glargine	Long Acting Insulin	Diabetes	Sanofi	$7.28 b
Avastin	Mab	Anti-VEGF-A	Colon Cancer etc.	Roche	$6.96 b
Herceptin	Mab	Anti-Her-2	Breast Cancer etc.	Roche	$6.79 b
Advair	Small Molecule	Ease constriction of the airways	Asthma etc.	GSK	$6.43 b
Crestor	Small Molecule	Inhibit HMG-CoA Reductase	High Cholesterol	AstraZeneca	$5.87 b
Neulasta	Pegylated Recombinant Protein	Boosting White Blood Cells	Infection and Neutropenia	Amgen	$5.86 b
Abilify	Small Molecule	Dopamine Agonist	Depression and Bipolar etc.	BMS	$5.27 b

Ready-to-use (R2U) cell line in the assay

- Ready-to-use cells have advantages over continuous cell culture
  - Eliminate routine laborious cell culture work
  - No drifting of cell quality due to cell passages
- The range of passage numbers of R2U cell bank have to be qualified
- Pfizer drug X is a recombinant factor which will stimulate dose-dependent cell proliferation in the assay
- Passage 7 R2U WCB had been used in original titration, DOE optimization and first method qualification
- Three additional Working Cell Banks (P5, P8 and P11) were made. P5 and P11 were used for R2U/method qualification

Assay plate map (after titration)

Plate 1

	1	2	3	4	5	6	7	8	9	10	11	12
A–H	Reference Standard a	Sample 1a	Sample 2a	Control a	Reference Standard a	Sample 1a	Sample 2a	Control a	Reference Standard a	Sample 1a	Sample 2a	Control a

Plate 2

	1	2	3	4	5	6	7	8	9	10	11	12
A–H	Reference Standard b	Sample 1b	Sample 2b	Control 2b	Reference Standard b	Sample 1b	Sample 2b	Control 2b	Reference Standard b	Sample 1b	Sample 2b	Control 2b

Plate 3

	1	2	3	4	5	6	7	8	9	10	11	12
A–H	Reference Standard c	Sample 1c	Sample 2c	Control 2c	Reference Standard c	Sample 1c	Sample 2c	Control 2c	Reference Standard c	Sample 1c	Sample 2c	Control 2c

Optimization DOE design with 16 runs

1st DOE HSP-130

Design: Custom Design
Criterion: I Optimal

Screening
Model
DOE Dialog

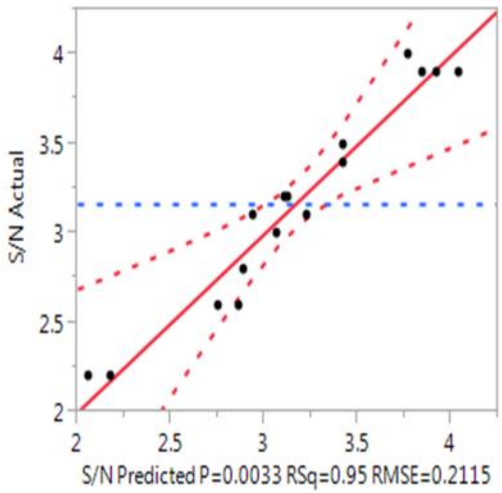
Columns (5/0)

	Cell	Stimulation	Incubation	S/N	EC50
1	7500	27	24	3.2	0.352
2	10000	30	24	3.9	0.388
3	10000	24	24	3	0.277
4	10000	24	16	3.1	0.307
5	5000	30	16	3.2	0.626
6	5000	30	24	2.6	0.4
7	10000	30	16	3.9	0.375
8	5000	27	20	2.8	0.395
9	7500	27	20	3.5	0.373
10	7500	27	20	3.4	0.351
11	5000	24	24	2.2	0.3
12	7500	30	20	4	0.328
13	5000	24	16	2.2	0.313
14	10000	27	20	3.9	0.406
15	7500	24	20	2.6	0.265
16	7500	27	16	3.1	0.275

Critical parameters in the assay

Response S/N

Actual by Predicted Plot

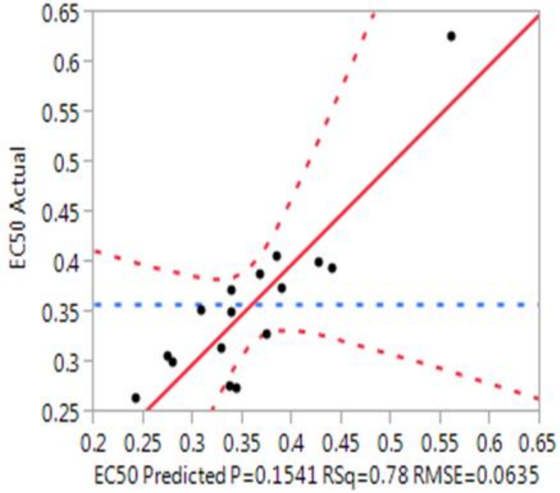


Sorted Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Cell(5000,10000)	0.48	0.066894	7.18	0.0004*
Stimulation(24,30)	0.45	0.066894	6.73	0.0005*
Incubation*Incubation	-0.253448	0.130283	-1.95	0.0997
Incubation(16,24)	-0.06	0.066894	-0.90	0.4043
Cell*Incubation	0.0625	0.07479	0.84	0.4353
Stimulation*Incubation	-0.0625	0.07479	-0.84	0.4353
Stimulation*Stimulation	-0.103448	0.130283	-0.79	0.4574
Cell*Stimulation	0.0375	0.07479	0.50	0.6339
Cell*Cell	-0.053448	0.130283	-0.41	0.6959

Response EC50

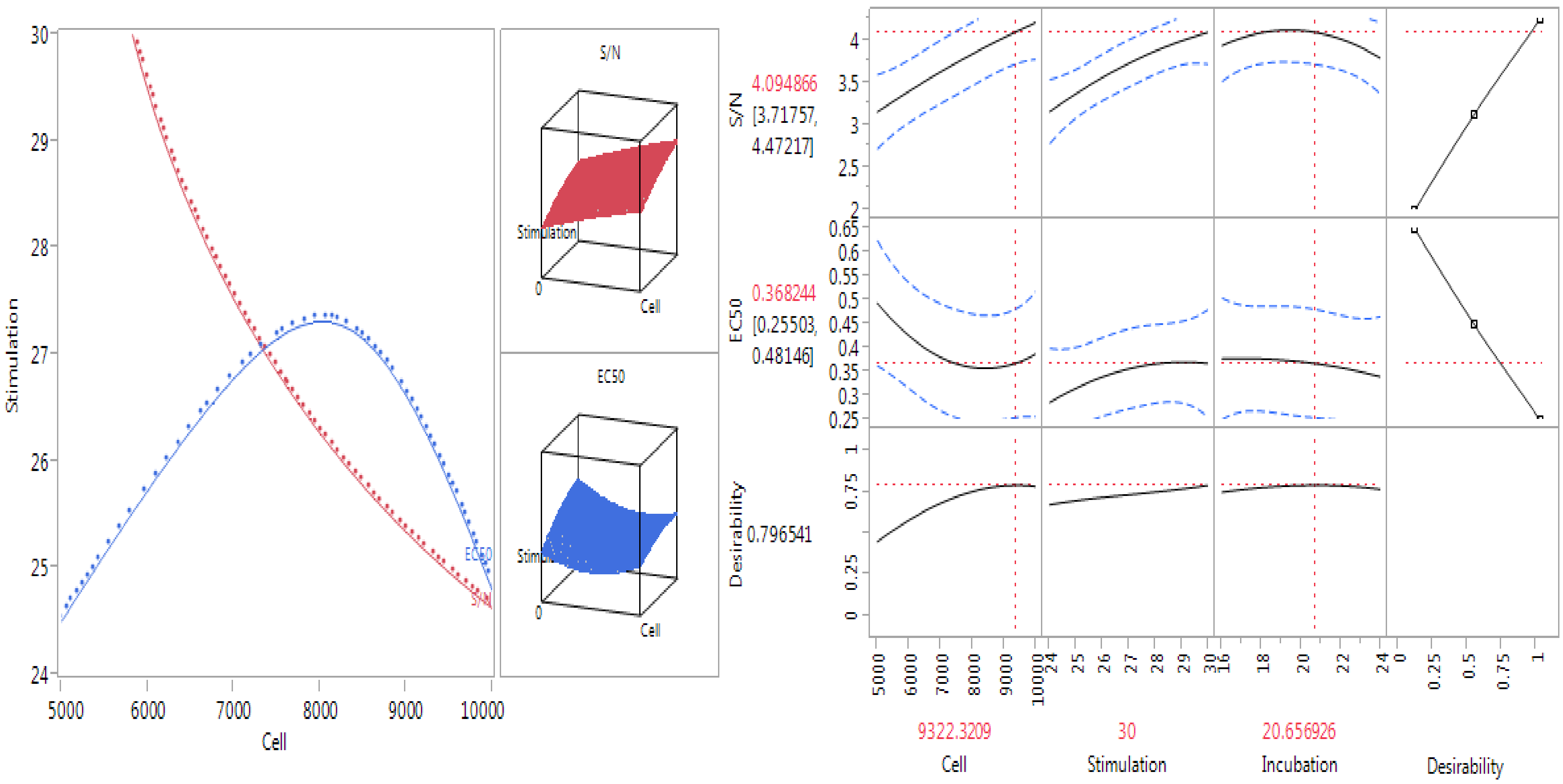
Actual by Predicted Plot



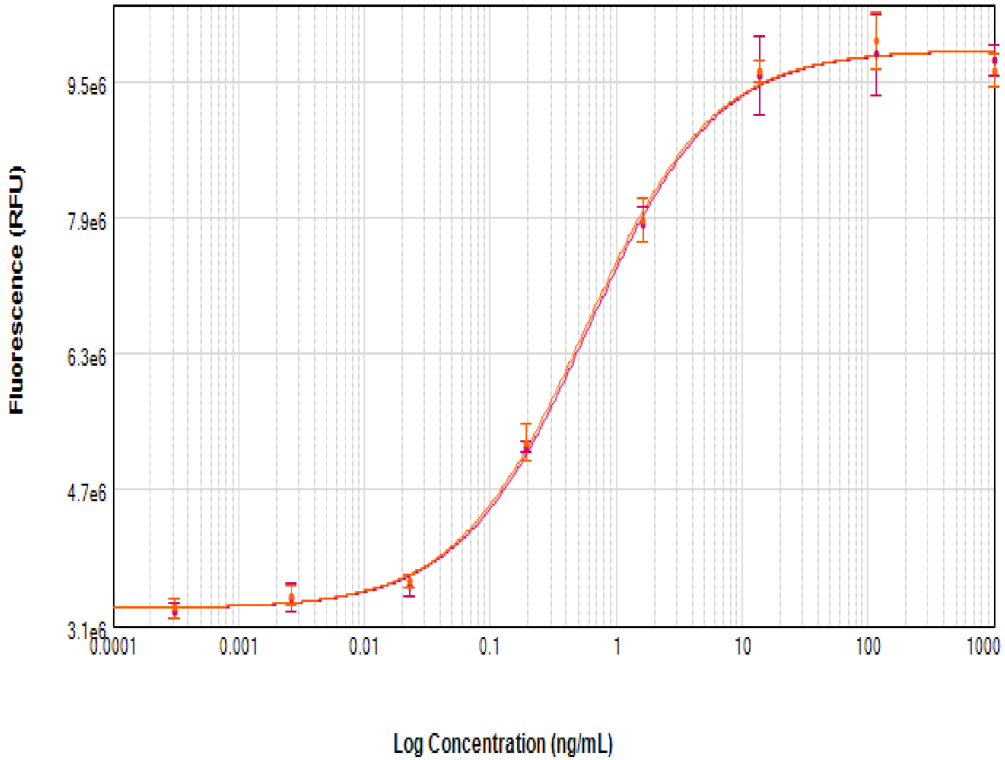
Sorted Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Stimulation(24,30)	0.0655	0.020073	3.26	0.0172*
Cell*Cell	0.0743966	0.039093	1.90	0.1057
Cell(5000,10000)	-0.0281	0.020073	-1.40	0.2111
Cell*Stimulation	-0.02925	0.022442	-1.30	0.2402
Cell*Incubation	0.02775	0.022442	1.24	0.2625
Stimulation*Incubation	-0.02125	0.022442	-0.95	0.3803
Incubation(16,24)	-0.0179	0.020073	-0.89	0.4069
Stimulation*Stimulation	-0.029603	0.039093	-0.76	0.4776
Incubation*Incubation	-0.012603	0.039093	-0.32	0.7581

DOE optimized final conditions

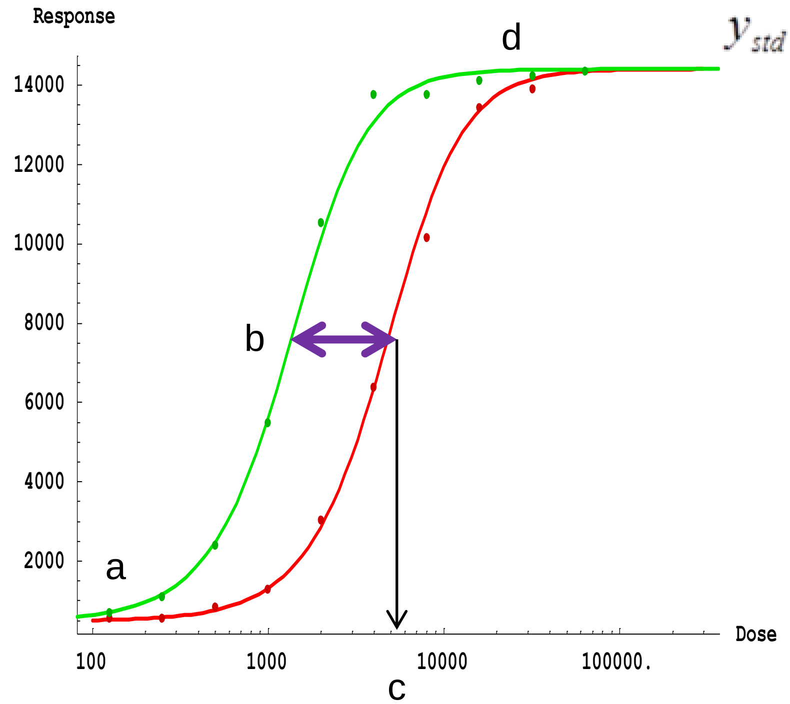


Finalized assay conditions



- Dilution scheme is 1:7 of 8-dose serial dilutions with starting concentration of 375 ng/mL
- 9500 cells/well; 30 hr stimulation and 21 hr substrate incubation

Full curve analysis



$$y_{std} = d + \frac{(a-d)}{1+\left(\frac{x}{c}\right)^{b}} \quad \longrightarrow \quad y_{ukn} = d + \frac{(a-d)}{1+\left(\frac{rx}{c}\right)^{b}}$$

- Parallelism Test
  - F-Test
  - Equivalence Test
- Relative Potency
  - the factor that the unknown dilution curve data would need to be multiplied by in order to shift the curve along the x-axis and overlay the standard curve

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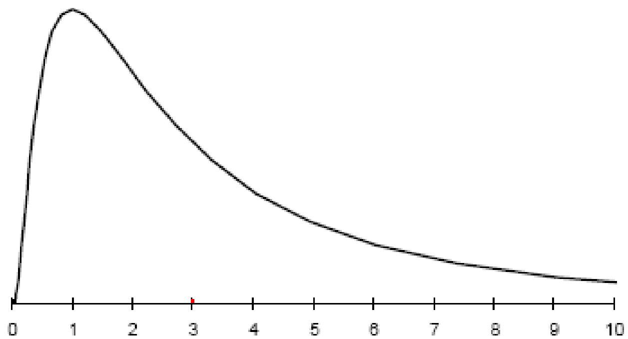
Full curve analysis using *F*-test

$$F = \frac{\frac{[RSSE(reduced) - RSSE(full)]}{DF}}{\frac{RSSE(full)}{(n-p)}}$$



F-test may factor out weighting and it is less sensitive

- If fit extremely well, the denominator will be very small, therefore, it will fail parallelism
- If fit not well, the denominator will be large and it may pass parallelism

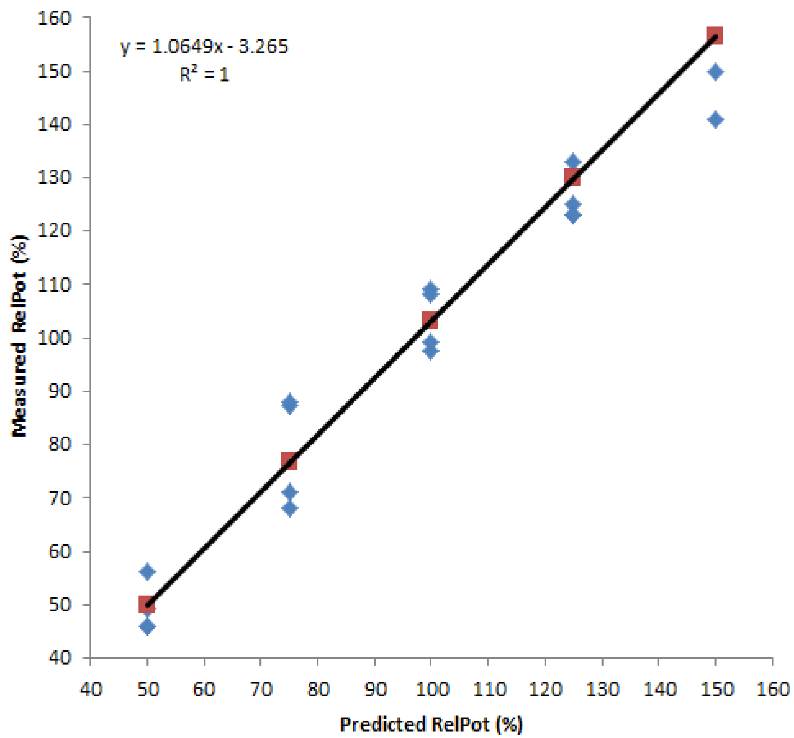
Initial assay acceptance criteria

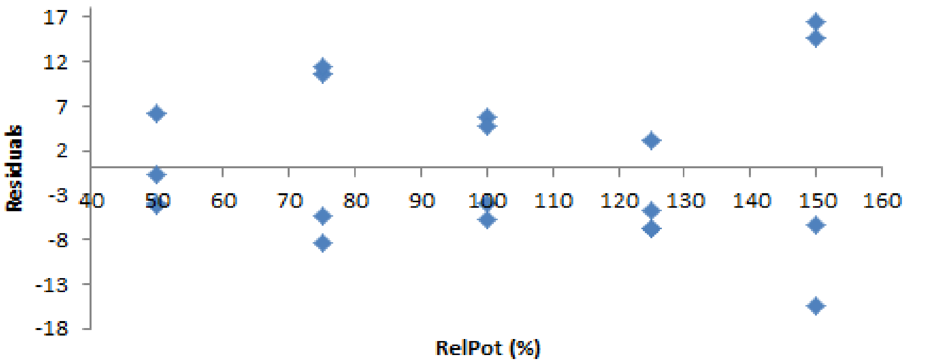
- Parallelism evaluation using *F*-test
  - *F* stat must be ≤ *F* critical value of 7.591 ($\alpha = 0.01$ with numerator DF of 3 and denominator DF of 8)
- Relative potency recovery
  - Sample and control relative potency must be within 70% to 130% to its target relative potency

Qualification: Accuracy, Repeatability and IP

Analyst	R2U Cell Bank	Plate	Sample				
			50	75	100	125	150
A	Passage 5	1-3	57.3	87.1	99.6	128	145
		4-6	56.3	87.5	92.8	121	143
		7-9	55.1	87.0	100	126	134
		Mean	56.2	87.2	97.5	125	141
		Repeatability %RSD	1.96	0.30	4.15	2.88	4.17
		Accuracy/Recovery (%)	112	116	97.5	100	93.8
	Passage 11	1-3	47.9	78.0	98.6	126	177
		4-6	40.2	67.2	103	129	172
		7-9	49.7	68.5	95.9	115	165
		Mean	45.9	71.2	99.2	123	171
		Repeatability %RSD	11.0	8.28	3.61	5.98	3.52
		Accuracy/Recovery (%)	91.9	95.0	99.2	98.7	114
C	Passage 5	1-3	49.1	67.7	111	126	163
		4-6	51.6	67.3	109	136	145
		7-9	47.0	69.6	108	137	144
		Mean	49.2	68.2	109	133	151
		Repeatability %RSD	4.68	1.80	1.40	4.57	7.10
		Accuracy/Recovery (%)	98.5	90.9	109	106	100
	Passage 11	1-3	46.3	80.0	113	123	174
		4-6	48.2	96.4	103	124	165
		7-9	43.5	87.4	107	123	180
		Mean	46.0	87.9	108	123	173
		Repeatability %RSD	5.14	9.34	4.67	0.47	4.36
		Accuracy/Recovery (%)	92.0	117	108	98.7	115
Intermediate Precision (P5, P11 for analyst A, C; n=4)			9.80	13.2	5.72	3.78	9.80

Qualification: Linearity and Specificity





Result	Sample			
	Matrix Only	Matrix spiked with Ref Std	Cytokine 1	mAb 1
RelPot (%)	No Response	99.7	No Response	No Response

Assay trending program (ATP)

- Trending program is very important
  - Increases assay consistency and quality
  - Decreases assay failure and repeat
- Establish trending program
  - Assay condition is finalized and accumulate enough assay results
  - Calculate control limits in control chart
- The Shewart control chart provides the tool to distinguish between the two types of variation in a process
  - Common cause variation (random)
  - Special cause variation (root cause)

Assay trending program (cont.)

A	B	C	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AI	AJ	
Assay Date	Analyst	Sample ID	Average Signal Noise	A Unconstrained	B Unconstrained	C Unconstrained	D Unconstrained	A	B	C	D	Max/Min	Individual RelPot (%)	Recovery Reportable Result (%)	95% CI Lower	95% CI Upper	Ftest Prob	F Test Stat	Note	Starting conc	
26-May-16	JH	CTRL 1	3.7	3.36E+06	0.823	0.474	1.23E+07	3.38E+06	0.836	0.52	1.24E+07	3.67	108		0.695	1.464	0.991	0.033	Qualification	375	CMB
26-May-16	JH	EPO	3.7	3.44E+06	78.04	0.055	3.38E+06	3.41E+06	0.862	0.594	1.24E+07	3.64	0		-4.06E-07	4.06E-07	0.796	0.342	Qualification	375	CMB
26-May-16	JH	Ranibizumab	3.7	3.36E+06	34.28	2.781	3.36E+06	3.45E+06	0.97	0.512	1.21E+07	3.51	0		-3.28E-05	3.28E-05	0.319	1.374	Qualification	375	CMB
2-Jun-16	GD	CTRL 1	3.5	3.22E+06	0.742	0.378	1.09E+07	3.15E+06	0.666	0.453	1.11E+07	3.52	110		0.7	1.502	0.486	0.891	Qualification	375	CMB
2-Jun-16	GD	CTRL 2	3.5	3.02E+06	0.608	0.446	1.13E+07	3.02E+06	0.628	0.422	1.11E+07	3.68	103		0.666	1.405	0.811	0.32	Qualification	375	CMB
2-Jun-16	GD	CTRL 3	3.5	3.06E+06	0.637	0.435	1.09E+07	3.11E+06	0.693	0.403	1.07E+07	3.44	106	106	0.702	1.409	0.253	1.651	Qualification	375	CMB
2-Jun-16	GD	Sample 1-1	3.5	3.19E+06	0.741	0.682	1.07E+07	3.14E+06	0.661	0.438	1.10E+07	3.50	55.8		0.36	0.756	0.273	1.559	Qualification	375	CMB
2-Jun-16	GD	Sample 1-2	3.5	3.14E+06	0.665	0.827	1.10E+07	3.09E+06	0.659	0.405	1.10E+07	3.56	51.4		0.353	0.674	0.958	0.1	Qualification	375	CMB
2-Jun-16	GD	Sample 1-3	3.5	3.16E+06	0.723	0.678	1.05E+07	3.16E+06	0.737	0.367	1.05E+07	3.32	55.7	109	0.429	0.684	0.968	0.082	Qualification	375	CMB
2-Jun-16	GD	Sample 2-1	3.5	3.07E+06	0.648	0.284	1.10E+07	3.08E+06	0.627	0.456	1.12E+07	3.64	148		0.75	2.204	0.918	0.164	Qualification	375	CMB
2-Jun-16	GD	Sample 2-2	3.5	3.76E+06	0.727	0.321	1.09E+07	3.35E+06	0.671	0.458	1.09E+07	3.25	174		0.57	2.915	0.503	0.852	Qualification	375	CMB
2-Jun-16	GD	Sample 2-3	3.5	3.15E+06	0.713	0.261	1.06E+07	3.15E+06	0.731	0.377	1.05E+07	3.33	152	105	1.177	1.853	0.782	0.362	Qualification	375	CMB
2-Jun-16	GD	CTRL 1	3.6	3.12E+06	0.683	0.46	1.11E+07	3.12E+06	0.691	0.42	1.09E+07	3.49	100		0.645	1.348	0.653	0.566	Qualification	375	CMB
2-Jun-16	GD	CTRL 2	3.6	3.10E+06	0.746	0.317	1.08E+07	3.10E+06	0.705	0.384	1.09E+07	3.52	115		0.802	1.505	0.819	0.308	Qualification	375	CMB
2-Jun-16	GD	CTRL 3	3.6	3.10E+06	0.641	0.392	1.15E+07	3.10E+06	0.575	0.367	1.16E+07	3.74	86.6	101	0.49	1.242	0.557	0.74	Qualification	375	CMB
2-Jun-16	GD	Sample 1-1	3.6	3.19E+06	0.751	0.769	1.09E+07	3.16E+06	0.725	0.402	1.08E+07	3.42	54.7		0.378	0.715	0.811	0.32	Qualification	375	CMB
2-Jun-16	GD	Sample 1-2	3.6	3.22E+06	0.734	0.751	1.08E+07	3.16E+06	0.7	0.398	1.09E+07	3.45	51.7		0.359	0.676	0.906	0.181	Qualification	375	CMB
2-Jun-16	GD	Sample 1-3	3.6	3.19E+06	0.645	0.873	1.14E+07	3.15E+06	0.58	0.373	1.16E+07	3.68	40	97.6	0.287	0.512	0.234	1.753	Qualification	375	CMB
2-Jun-16	GD	Sample 2-1	3.6	3.06E+06	0.698	0.249	1.09E+07	3.09E+06	0.699	0.394	1.08E+07	3.50	161		0.983	2.228	0.942	0.126	Qualification	375	CMB
2-Jun-16	GD	Sample 2-2	3.6	3.07E+06	0.682	0.265	1.10E+07	3.09E+06	0.676	0.402	1.10E+07	3.56	150		0.928	2.08	0.993	0.028	Qualification	375	CMB
2-Jun-16	GD	Sample 2-3	3.6	3.00E+06	0.622	0.294	1.14E+07	3.04E+06	0.566	0.34	1.15E+07	3.78	101	91.6	0.626	1.396	0.433	1.02	Qualification	375	CMB
2-Jun-16	JH	CTRL 1	3.5	3.08E+06	0.651	0.722	1.21E+07	3.18E+06	0.711	0.714	1.17E+07	3.68	114		0.722	1.561	0.151	2.328	Qualification	375	CMB
2-Jun-16	JH	CTRL 2	3.5	3.18E+06	0.695	0.606	1.13E+07	3.20E+06	0.739	0.562	1.13E+07	3.53	91.4		0.606	1.222	0.701	0.487	Qualification	375	CMB
2-Jun-16	JH	CTRL 3	3.5	3.04E+06	0.732	0.661	1.11E+07	3.09E+06	0.705	0.803	1.11E+07	3.59	117	107	0.791	1.542	0.797	0.341	Qualification	375	CMB
2-Jun-16	JH	Sample 1-1	3.5	3.27E+06	0.833	1.263	1.14E+07	3.27E+06	0.81	0.622	1.14E+07	3.49	48.9		0.371	0.607	0.948	0.116	Qualification	375	CMB
2-Jun-16	JH	Sample 1-2	3.5	3.17E+06	0.758	1.308	1.16E+07	3.19E+06	0.774	0.592	1.14E+07	3.57	47.6		0.332	0.621	0.898	0.193	Qualification	375	CMB
2-Jun-16	JH	Sample 1-3	3.5	3.19E+06	0.747	1.693	1.12E+07	3.17E+06	0.714	0.873	1.12E+07	3.53	53.6	100	0.382	0.69	0.606	0.649	Qualification	375	CMB
2-Jun-16	JH	Sample 2-1	3.5	3.16E+06	0.725	0.407	1.17E+07	3.22E+06	0.754	0.655	1.15E+07	3.57	168		1.213	2.145	0.705	0.48	Qualification	375	CMB
2-Jun-16	JH	Sample 2-2	3.5	3.16E+06	0.82	0.386	1.12E+07	3.19E+06	0.803	0.545	1.13E+07	3.54	135		0.908	1.795	0.951	0.111	Qualification	375	CMB
2-Jun-16	JH	Sample 2-3	3.5	3.14E+06	0.783	0.52	1.09E+07	3.14E+06	0.726	0.784	1.10E+07	3.50	142	98.9	1.012	1.823	0.652	0.568	Qualification	375	CMB
2-Jun-16	JH	CTRL 1	3.8	3.13E+06	0.686	0.696	1.20E+07	3.13E+06	0.656	0.723	1.24E+07	3.96	87.8		0.44	1.315	0.614	0.634	Qualification	375	CMB
2-Jun-16	JH	CTRL 2	3.8	3.00E+06	0.559	1.108	1.31E+07	3.08E+06	0.62	0.854	1.27E+07	4.12	92.9		0.478	1.38	0.709	0.474	Qualification	375	CMB
2-Jun-16	JH	CTRL 3	3.8	3.22E+06	0.692	0.624	1.13E+07	3.22E+06	0.734	0.554	1.13E+07	3.51	89.4	90.0	0.595	1.194	0.762	0.393	Qualification	375	CMB
2-Jun-16	JH	Sample 1-1	3.8	3.20E+06	0.712	1.495	1.21E+07	3.16E+06	0.664	0.768	1.25E+07	3.96	44.7		0.274	0.619	0.711	0.47	Qualification	375	CMB
2-Jun-16	JH	Sample 1-2	3.8	3.11E+06	0.718	1.345	1.20E+07	3.12E+06	0.696	0.688	1.22E+07	3.91	46.9		0.296	0.642	0.91	0.176	Qualification	375	CMB
2-Jun-16	JH	Sample 1-3	3.8	3.20E+06	0.763	1.296	1.16E+07	3.21E+06	0.773	0.588	1.14E+07	3.55	49	93.7	0.338	0.643	0.652	0.567	Qualification	375	CMB
2-Jun-16	JH	Sample 2-1	3.8	3.14E+06	0.68	0.467	1.20E+07	3.14E+06	0.659	0.715	1.23E+07	3.92	131		0.725	1.885	0.416	1.065	Qualification	375	CMB

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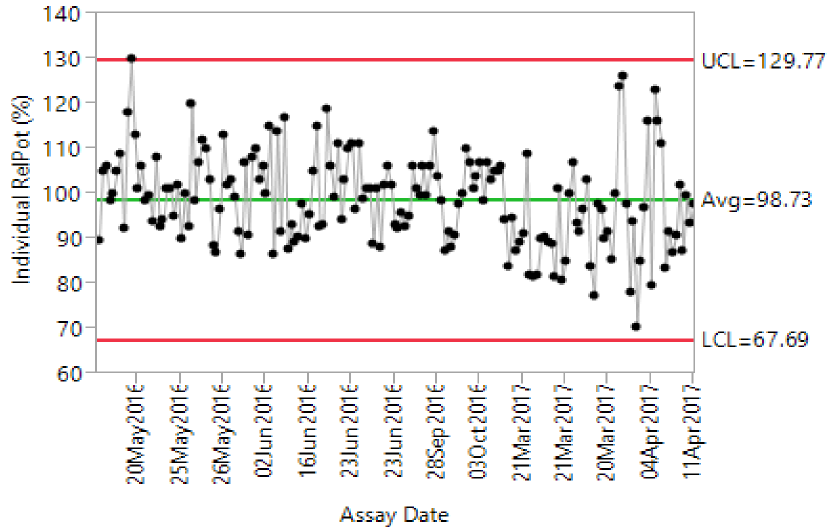
Research and Development

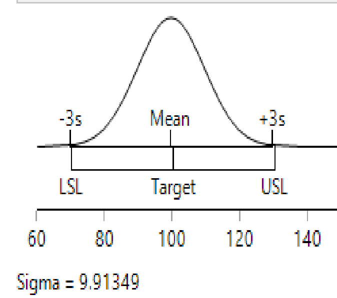
System suitability/Control Trending



Capability Analysis

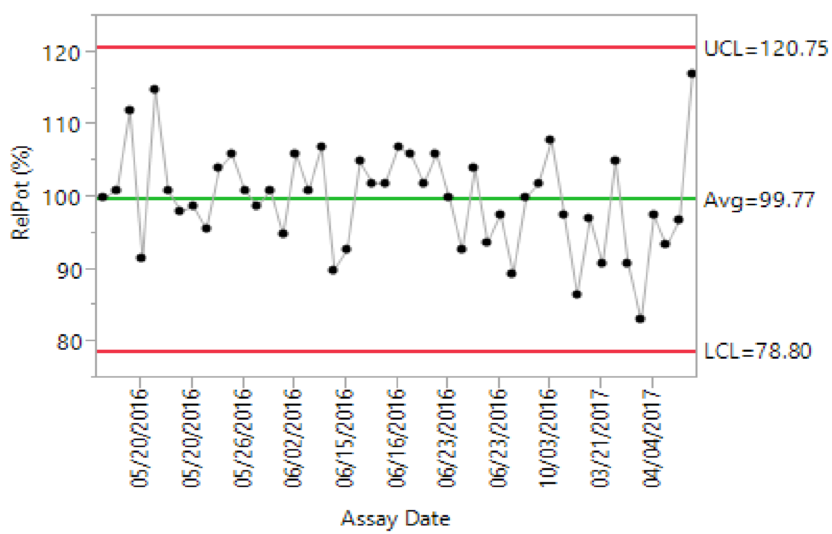
Specification	Value	Portion	% Actual
Lower Spec Limit	70	Below LSL	0.0000
Spec Target	100	Above USL	0.0000
Upper Spec Limit	130	Total Outside	0.0000

Long Term Sigma



Capability	Index	Lower CI	Upper CI
CP	1.009	0.896	1.121
CPK	0.986	0.864	1.107
CPM	1.006	0.898	1.121
CPL	0.986	0.864	1.107
CPU	1.032	0.905	1.158

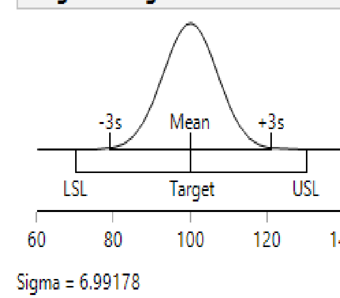
Portion	Percent	PPM	Sigma Quality
Below LSL	0.1554	1554.2663	4.457
Above USL	0.0982	982.2049	4.596



Capability Analysis

Specification	Value	Portion	% Actual
Lower Spec Limit	70	Below LSL	0.0000
Spec Target	100	Above USL	0.0000
Upper Spec Limit	130	Total Outside	0.0000

Long Term Sigma



Capability	Index	Lower CI	Upper CI
CP	1.430	1.139	1.721
CPK	1.419	1.114	1.725
CPM	1.430	1.154	1.736
CPL	1.419	1.114	1.723
CPU	1.441	1.131	1.749

Portion	Percent	PPM	Sigma Quality
Below LSL	0.0010	10.2903	5.758
Above USL	0.0008	7.6958	5.823

Full curve analysis using equivalence test

- Similarity is demonstrated when:

  a_t/a_s; b_t/b_s; and d_t/d_s fall within preset acceptance criteria

- For one bioassay example:

Acceptance Parameter	Sample Size (N)	Mean	Standard Deviation	LAL	UAL
R^2	680	0.991	0.007	0.97	1.00
a_S/a_R	452	0.984	0.042	0.85	1.15
d_S/d_R	452	0.983	0.983	0.80	1.20
b_S/b_R	452	0.956	0.163	0.55	1.45

Amgen, Inc., Frank Ye 2005

Concept of equivalence test

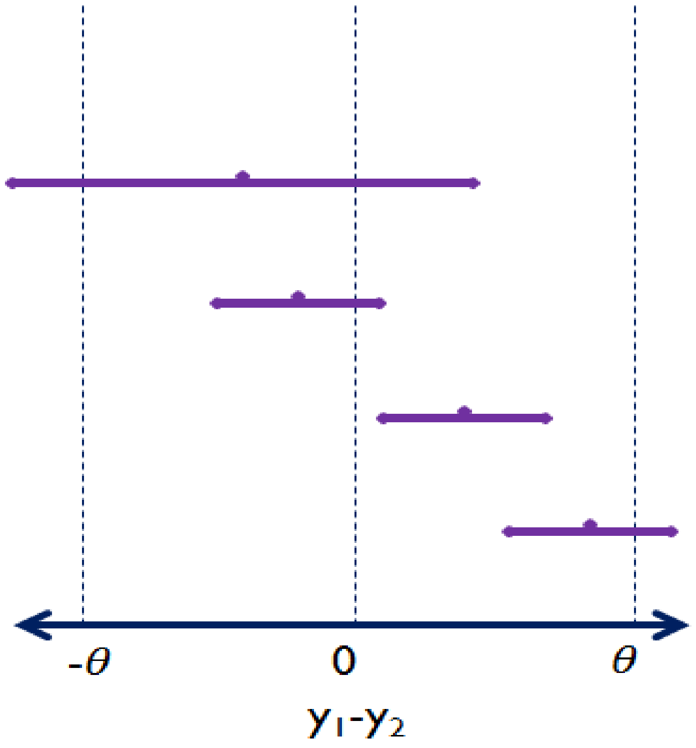
- Switch the null and alternative hypothesis
- Set up data driven pre-determined goalposts
- Equivalence test: two-one sided t-test (TOST)
- The two null hypothesis will be rejected if

$$T_2 \leq t_{\alpha,\nu} \quad \textit{or} \quad T_1 \geq t_{1-\alpha,\nu}$$

$$T_2 = \frac{(\bar{\mu}_1 - \bar{\mu}_2) - \theta}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \qquad T_1 = \frac{\theta - (\bar{\mu}_1 - \bar{\mu}_2)}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

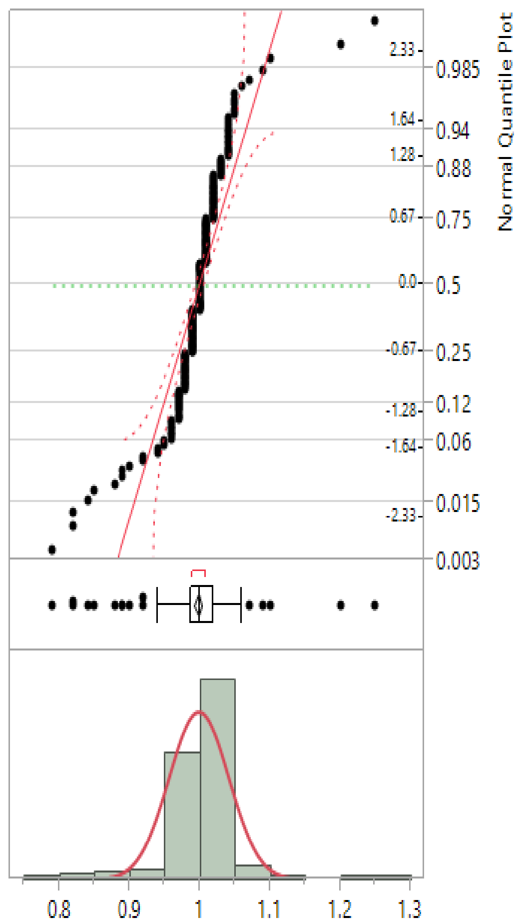
θ *is the goalpost which is predetermined*

Comparison of traditional hypothesis test and equivalence test



Scenario	Simple *t*-test	Equivalence test
1	Equivalent	Not equivalent
2	Equivalent	Equivalent
3	Not equivalent	Equivalent
4	Not equivalent	Not equivalent

Assessment of tolerance interval for lower asymptote



Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	0.9981102	0.992859	1.0033615
Dispersion	σ	0.0424959	0.0390937	0.0465517

-2log(Likelihood) = -884.619940417157

Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.778798	<.0001*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Custom Quantiles

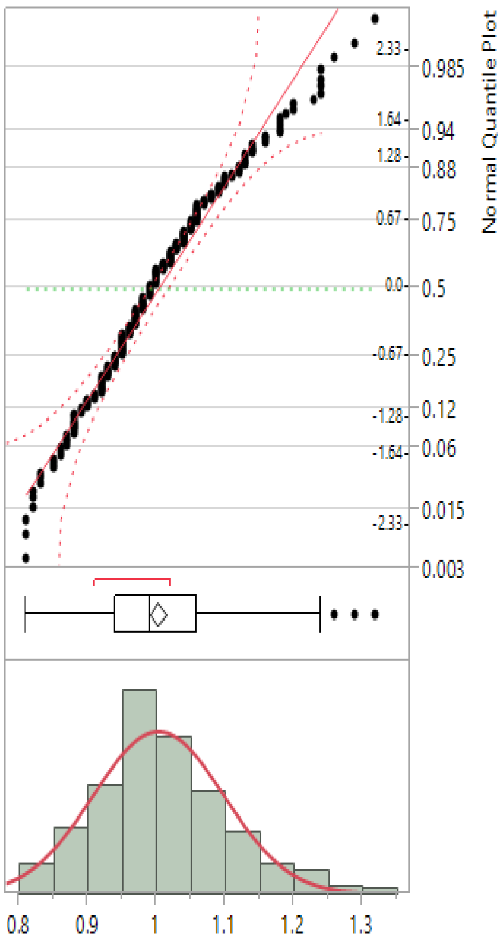
Quantiles

Quantile	Estimate	Lower 95%	Upper 95%	Actual Coverage
0.5%	0.79825	0.79	1.25	72.01
50%	1	1	1	95.51
99.5%	1.23625	0.79	1.25	72.01

Smoothed Empirical Likelihood Quantiles

Quantile	Estimate	Lower 95%	Upper 95%
0.5%	0.81754	0.78853	0.84794
50%	1.00019	0.99821	1.00232
99.5%	1.20145	1.07212	1.25147

Assessment of tolerance interval for slope



Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	1.0020866	0.9903283	1.013845
Dispersion	σ	0.0951553	0.0875373	0.1042368

-2log(Likelihood) = -475.11993027204

Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.976338	0.0003*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Custom Quantiles

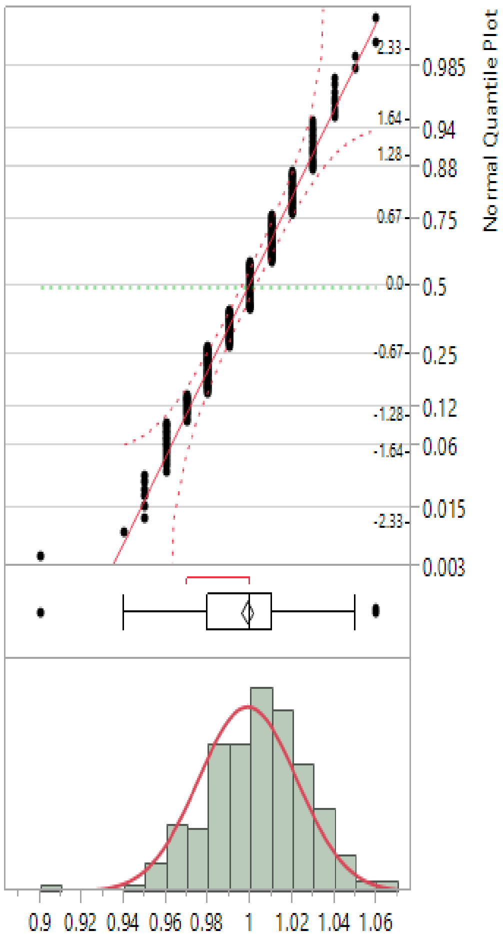
Quantiles

Quantile	Estimate	Lower 95%	Upper 95%	Actual Coverage
0.5%	0.81	0.81	1.32	72.01
50%	0.99	0.98	1	95.51
99.5%	1.31175	0.81	1.32	72.01

Smoothed Empirical Likelihood Quantiles

Quantile	Estimate	Lower 95%	Upper 95%
0.5%	0.80732	0.80173	0.81609
50%	0.99133	0.97996	1.00369
99.5%	1.29534	1.24719	1.32541

Assessment of tolerance interval for upper asymptote



Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	0.9984646	0.9955629	1.0013662
Dispersion	σ	0.0234817	0.0216018	0.0257227

-2log(Likelihood) = -1185.95943688135

Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.976098	0.0003*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Custom Quantiles

Quantiles

Quantile	Estimate	Lower 95%	Upper 95%	Actual Coverage
0.5%	0.911	0.9	1.06	72.01
50%	1	1	1	95.51
99.5%	1.06	0.9	1.06	72.01

Smoothed Empirical Likelihood Quantiles

Quantile	Estimate	Lower 95%	Upper 95%
0.5%	0.93866	0.89865	0.94845
50%	0.99982	0.99786	1.00165
99.5%	1.05923	1.04368	1.06179

AC based on non-parametric tolerance interval

- The distribution of parameters of a, b, d are not normal
- Data transformations are not normal distributed either
- The ratios of parameters a, b, d to reference standard were chosen for acceptance criteria set up
- Non-parametric tolerance intervals for a, b, d were calculated from 267 data points generated using reference standard material
  - addressing pure analytical variation

Ratio of parameter	Acceptance Range
a/a’	0.85 – 1.07
b/b’	0.82 – 1.25
d/d’	0.95– 1.04

Reportable results control strategy

- Arithmetic mean of three independent assay results
  - %RSD criterion among three independent assay results (e.g. ≤20% RSD)
- Weighted mean of three independent assay results
  - %RSD criterion among three independent assay results (e.g. ≤20% RSD)
- Geometric mean of three independent assay results
  - Confidence interval criterion for three independent assay results

Weighted mean

- The weighted mean is calculated as

$$\overline{M} = \frac{\sum_{i=1}^{3}(W_i M_i)}{\sum_{i=1}^{3} W_i}$$

- The individual result with less variation will have more influence on the final reportable result than the individual result with larger variation
- An acceptance criterion for the weighted mean is determined by %RSD the same as for an arithmetic mean

Geometric mean

- The data log transformation generates normal distributed results
- Therefore, geometric mean addresses better for skewed results and is closer to true value
- The Geometric Mean calculation

$$Geometric\ Mean = e^{\ln RP1 + \ln RP2 + \ln RP3}$$

- Geometric Mean is controlled by the confidence interval (e.g. 75% to 133%)

Reportable result calculation using geometric mean

Sample	Result		
	Plate 1	Plate 2	Plate 3
1	56.7	52.3	46.9
2	152	158	168
3	69.5	70.2	74.7
4	126	125	135
5	101	105	108
6	106	110	104

Sample	Plate 1	Plate 2	Plate 3	Average	SD	n	LL	UL	Antilog				
									Reportable Result (geometric Mean)	LL	UL	Relative 95% CI LL	Relative 95% CI UL
1	4.0377742	3.9569964	3.848017675	3.9475961	0.0952269	3	3.7110394	4.1841528	51.810669	40.896292	65.637868	78.934113	126.68794
2	5.0238805	5.062595	5.123963979	5.0701465	0.0504672	3	4.9447789	5.1955141	159.19765	140.4398	180.4609	88.217256	113.35651
3	4.2413268	4.2513483	4.313480092	4.2687184	0.0390873	3	4.1716202	4.3658166	71.430031	64.820388	78.71365	90.746689	110.19686
4	4.8362819	4.8283137	4.905274778	4.8566235	0.0423212	3	4.7514918	4.9617552	128.58928	115.75684	142.8443	90.020594	111.08569
5	4.6151205	4.6539604	4.682131227	4.650404	0.0336466	3	4.5668212	4.7339868	104.62725	96.237704	113.74816	91.981491	108.71752
6	4.6634391	4.7004804	4.644390899	4.6694368	0.0285217	3	4.598585	4.7402886	106.63767	99.343645	114.46723	93.159996	107.34221

Finalized acceptance criteria

- Assay acceptance criteria
  - Control sample must pass equivalence test for parallelism
  - The relative potency of control sample must be within the range of 70% to 130% of the reference standard
  - The upper/lower asymptote in constrained model must be equal or larger than 3
- Sample acceptance criteria
  - Sample must pass equivalence test for parallelism
  - The relative potency of sample must be within the qualified range of 50% to 150% of the reference standard
  - The upper/lower asymptote in constrained model must be equal or larger than 3
- 90% confidence interval of the final reportable result must be within 75% to 133%

Summary

- The cell-based potency assay and R2U cell banks have been successfully optimized and qualified
- Both the parallelism evaluation and the final reportable result have been adopted according to the recommendations from the most recent USP chapter 1033
- The acceptance criteria have been set up based on accumulated historical data
- Cell-based potency assay is a critical analytical test in CMC package. Traditional method has to be evolved to meet the higher regulatory and industry standards

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