Piramal
Pharma Solutions

DESIGN OF EXPERIMENT (DOE) IN ASSESSING ROBUSTNESS AND FOR QUALIFICATION OF A CELL BASED POTENCY ASSAY.

ABSTRACT

The method was previously qualified in a thaw for use format with a precision of 10% and bias/ accuracy of 13% over the linear range 50%, to 200%. The method utilised standard parameters allowing the anchorage dependent cells to recover overnight prior to adding the drug. At a later stage the assay format was further developed with drug being added to cells on the same day as cell thaw. Concomitant with re-qualification the robustness of the methodology was also assessed by Design of Experiment (DoE), the data generated being used to support the planned validation activities. This paper seeks to discuss salient points in establishing robustness of the cell based potency assay, with particular reference to activities required for phase specific qualification and for validation using DoE.

INTRODUCTION

A key milestone in bioassay development is the execution of robustness studies, usually conducted prior to validation and the testing of commercial product (1). It is important that the bioassay is sufficiently robust to determine manufacturing consistency and to achieve this a bioassay may require further development and refinement throughout a product's lifecycle (). DoE is a tool that can be used to systemically evaluate multiple variables and can also provide an assessment of the impact from the interaction of related experimental conditions (2). DoE has been applied to cell based bioassay robustness and qualification studies (3,4,5), the ultimate aim of such studies is to define the "design space" to ensure that the bioassay methodology is fit to support commercial manufacture (1).

DoE or a QbD approach to analytical validation is increasingly being advocated for bioassays (1,2). Indeed, USP Chapter <1033> Biological Assay Validation provides an example of a 3-factor DoE approach to validation (6). The factors in this nested design were analyst, cell preparation and reagent lot which represent key variables that would change over the product's lifecycle. In the USP approach individual results are used to determine the format that has acceptable precision and method capability (Cpm), that will meet the release specification and to mitigate the risk of generating an Out Of Specification (6). We have previously used a two factor (operator, cell bank) design for validation of a bioassay. In this paper we report the use of DoE in establishing the robustness of the bioassay "design space", with the centre point

data being used for qualification and for determining the requirements to support validation of the bioassay using DoE.

METHODOLOGY AND EXPERIMENTAL DESIGN

The cell based assay, in a thaw-for-use format, was developed to measure the specific cytotoxic activity of an antibody drug conjugate towards a cell line expressing the target antigen. Cells were seeded into three 96 well plates at a nominal density of 3000 cells per well, and within 4 hours an 11-point dose dilution series of drug was added to the wells. The activity of the drug was determined after 4 days exposure using Alamar blue as metabolic reagent.

The methodology utilised optimised assay conditions; not only the number of cells per well, but recovery period prior to adding drug, the duration of drug exposure and Alamar blue development time. The objective of the current study was to assess the robustness of the methodology and to qualify the methodology for determining the relative potency of the antibody drug conjugate.

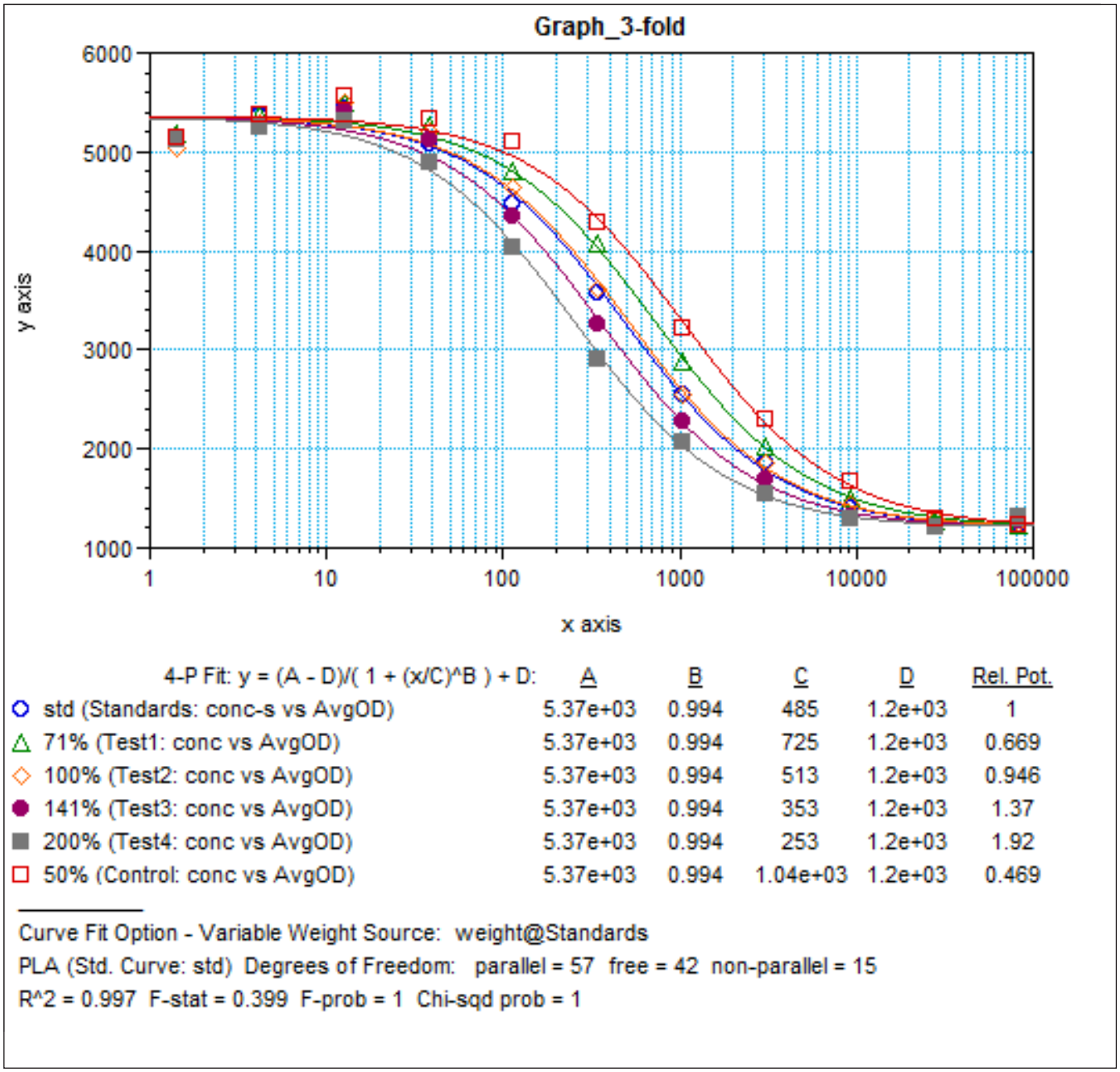
There were 8 factors (cell bank, cell number, dose dilution series, cell recovery time, drug exposure time, Alamar blue concentration, Alamar blue development time and FBS concentration) considered to be important in assessing the robustness of the methodology. The goal of the experimental design was to test all of the variables, testing for interactions and ifrequired allowing for parameter optimisation. With 8 factors the fractional factorial design becomes very large and difficult to manage and a different approach was taken to incorporate all 8 factors into the study.

Alamar blue is a non-destructive metabolic reagent and at each event assay plates were read at both the upper and lower limit time points. Five mock potency samples (50%, 71%, 100% 141% & 200%) were independently prepared using either a 2.7-fold or 3-fold dilution series and analysed at each event. This provided sample replication at events and was used to assess the robustness of the dose dilutional series. The remaining 6 factors were studied in a fractional factorial design where cell bank was a categorical variable (Table 1). Four centre points were incorporated into the design and a further four centre points were generated in the assessment of environmental factors (temperature and %CO2) and of reagent stability. Two analyst conducted a similar number of events.

A typical example of the dose dilution curves is shown in Figure 1 and for the purpose of this report the parallel line analysis and relative potency calculations

is presented as a single calculation. There is good agreement in EC50 and relative potency of the reference standard and five mock potency samples, and this will be discussed in more detail below.

Figure 1: Dilutional response using a 3-fold dilution series



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	Rel. Pot.
std (Standards: conc-s vs AvgOD)	5.37e+03	0.994	485	1.2e+03	1
71% (Test1: conc vs AvgOD)	5.37e+03	0.994	725	1.2e+03	0.669
100% (Test2: conc vs AvgOD)	5.37e+03	0.994	513	1.2e+03	0.946
141% (Test3: conc vs AvgOD)	5.37e+03	0.994	353	1.2e+03	1.37
200% (Test4: conc vs AvgOD)	5.37e+03	0.994	253	1.2e+03	1.92
50% (Control: conc vs AvgOD)	5.37e+03	0.994	1.04e+03	1.2e+03	0.469

Curve Fit Option - Variable Weight Source: weight@Standards
PLA (Std. Curve: std) Degrees of Freedom: parallel = 57 free = 42 non-parallel = 15
R^2 = 0.997 F-stat = 0.399 F-prob = 1 Chi-sqd prob = 1

ASSAY QUALIFICATION

The primary assumption underlying potency assays is that test sample will act as a dilution of the reference standard preparation and that mock potency samples are able to generate an increase or decrease in response proportional to the expected concentration (see Figure 1) (20). It is becoming increasingly important to incorporate factors which may be a source of assay variability (ie. plate location, operator, day, etc) and the use of experimental design is advocated for bioassay validation (5). A multi-operator design, conducted over several days, with replication at each event will provide estimates of precision (intermediate precision), accuracy/bias, linearity and the range of the methodology (5).

It is generally recognised that potency validation/qualification should use independent dilutions. A 1.41-fold geometric dilution series gives five mock potency samples at 200%, 141% 100%, 71% and 50% potency. The activity (relative potency) is determined relative to the standard preparation (100%), following parallel line analysis (PLA) of the non-linear dose response curves (4-7). It should be recognized that relative potency data are not normally distributed and log transformation provides data that are more appropriate to statistical analysis (5)

In the present study the centre point data was used for assay qualification against predetermined acceptancecriteria, and this will be compared to the robustness data generated from the experimental design.

Table 1: DoE used for robustness assessment

std.no	Run.no	Cell number	Cell Time (Hrs)	Drug Time (Hrs)	Alamar Vol.	%FBS	Cell Bank	Dilution	Sample Position				
									1	2	3	4	5
2	1	4000	1	88	15	12	A	3fold 2.7fold	50 71	71 100	100 141	141 200	200 50
5	2	2000	1	96	15	12	B	3fold 2.7fold	100 71	141 100	200 141	50 200	71 50
20	3	3000	2.5	92	20	10	B	3fold	50 200	71 50	100 71	141 100	200 141
3	4	2000	4	88	15	12	B	3fold 2.7fold	141 100	200 141	50 200	71 50	100 71
1	5	2000	1	88	15	8	A	3fold 2.7fold	71 50	100 71	141 100	200 141	50 200
8	6	4000	4	96	15	12	A	3fold 2.7fold	200 141	50 200	71 50	100 71	141 100
6	7	4000	1	96	15	8	B	3fold 2.7fold	100 71	141 100	200 141	50 200	71 50
13	8	2000	1	96	25	12	A	3fold 2.7fold	50 200	71 50	100 71	141 100	200 141
10	9	4000	1	88	25	12	B	3fold 2.7fold	141 100	200 141	50 200	71 50	100 71
18	10	3000	2.5	92	20	10	A	3fold	71 50	100 71	141 100	200 141	50 200
9	11	2000	1	88	25	8	B	3fold 2.7fold	200 141	50 200	71 50	100 71	141 100
15	12	2000	4	96	25	8	B	3fold 2.7fold	100 71	141 100	200 141	50 200	71 50

std.no	Run.no	Cell number	Cell Time (Hrs)	Drug Time (Hrs)	Alamar Vol.	%FBS	Cell Bank	Dilution	Sample Position				
									1	2	3	4	5
16	13	4000	4	96	25	12	B	3fold 2.7fold	50 200	71 50	100 71	141 100	200 141
19	14	3000	2.5	92	20	10	BA	3fold	141 100	200 141	50 200	71 50	100 71
12	15	4000	4	88	25	8	A	3fold 2.7fold	71 50	100 71	141 100	200 141	50 200
11	16	2000	4	88	25	12	A	3fold 2.7fold	200 141	50 200	71 50	100 71	141 100
14	17	4000	1	96	25	8	A	3fold 2.7fold	100 71	141 100	200 141	50 200	71 50
4	18	4000	4	88	15	8	B	3fold 2.7fold	200 141	50 200	71 50	100 71	141 100
17	19	3000	2.5	92	20	10	B	3fold	141 100	200 141	50 200	71 50	100 71
7	20	2000	4	96	15	8	A	3fold 2.7fold	50 200	71 50	100 71	141 100	200 141

METHODOLOGY AND EXPERIMENTAL DESIGN

A sample matrix was used such that the two operators analysed the five mock potency samples in different plate locations over the eight independent events using two working cell banks (Table 1). A second set of independently prepared mock potency samples and standard were analysed to provide the replication for determining intermediate precision (5). Intermediate precision, dilutional linearity and accuracy/bias were determined using the log10 derivative of the relative potency data (5). The centre point data are presented in Table 2.

Table 2: Centre point data used for qualification of the Cell Killing Assay

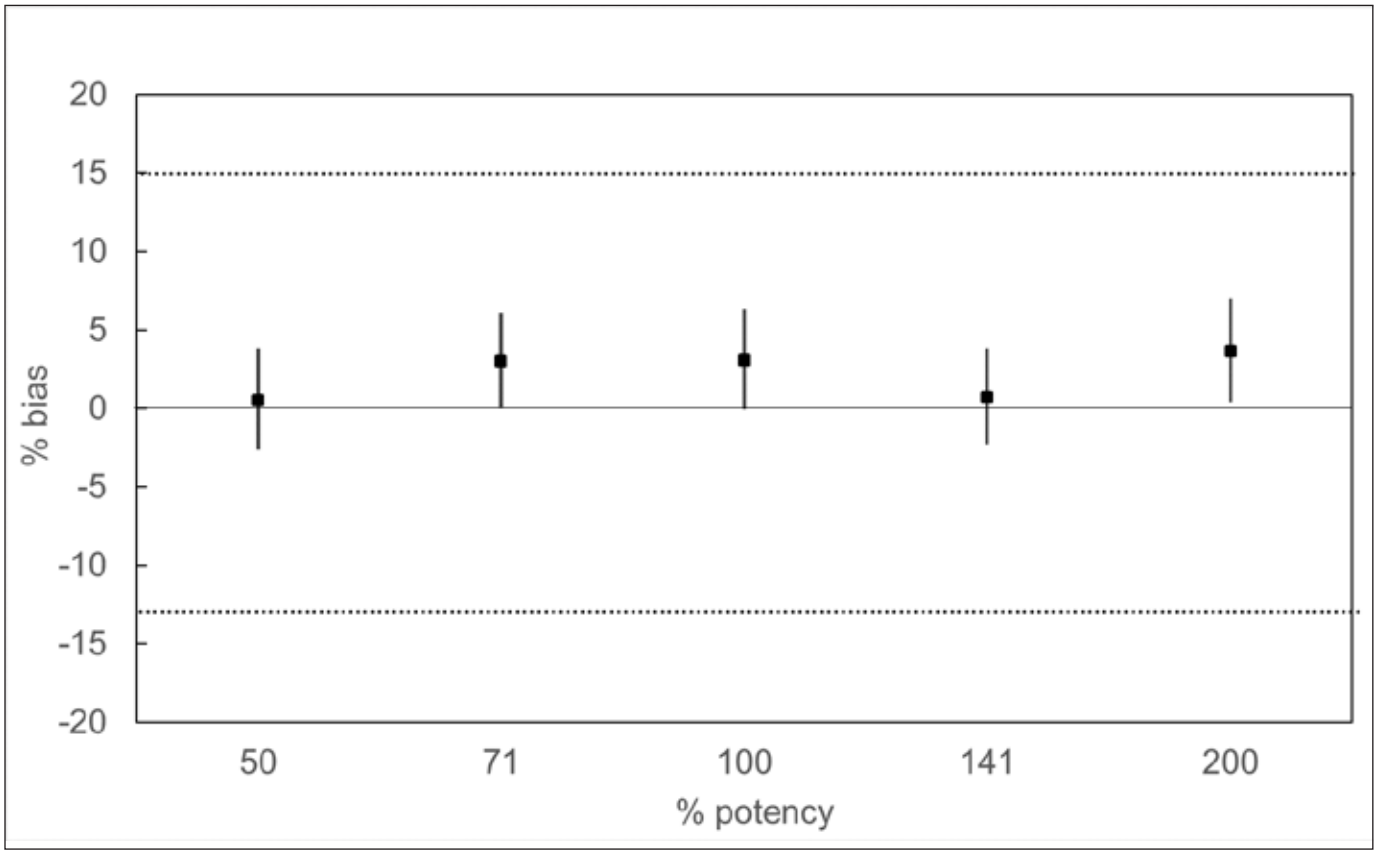
	Potency				
Event	50	71	100	141	200
1	45.0 52.6	62.5 79.2	97.1 107.6	159.4 151.6	194.1 227.8
2	51.6 51.3	67.5 68.8	93.6 106.0	136.0 151.7	214.9 217.0
3	57.5 51.6	75.6 78.8	110.0 109.4	148.6 146.3	201.7 204.8
4	50.9 52.2	76.6 73.6	113.0 103.0	155.5 140.9	210.6 214.9
5	47.6 49.7	70.7 77.4	96.5 107.6	131.7 137.5	209.4 222.3
6	44.5 51.8	79.3 73.3	96.3 116.2	149.6 144.7	175.1 213.4
7	56.5 49.7	75.0 72.7	103.5 97.2	128.8 135.6	224.8 203.4
8	45.3 48.5	67.7 74.1	91.5 104.8	128.0 131.7	180.8 209.5
mean	**50.3**	**73.1**	**103.1**	**142.0**	**207.3**
Lower 90%	**48.7**	**71.0**	**99.9**	**137.7**	**200.8**
Upper 90%	**51.9**	**75.3**	**106.3**	**146.4**	**214.0**

The log10 derivative was used to calculate the geometric mean (GM), 90% confidence interval of the geometric mean, mean relative bias (RB) and the 90% confidence interval of the mean relative bias according to <USP 1033> (Table 3). The %relative bias data is shown as a plot of mean bias ( ) with the 90% confidence interval of the mean (represented by the bars), and demonstrates equally variability across the mock potency samples centred at zero for the 50%, 71%, 100% and 141% with no obvious pattern across the potency range (Figure 2). For the 200% mock potency sample there was a small positive bias which was within the acceptance criteria of +15 to -13% (dotted line) indicative of accuracy in the relative potency measurement over the range of samples tested.

Table 3: Summary of the qualification data

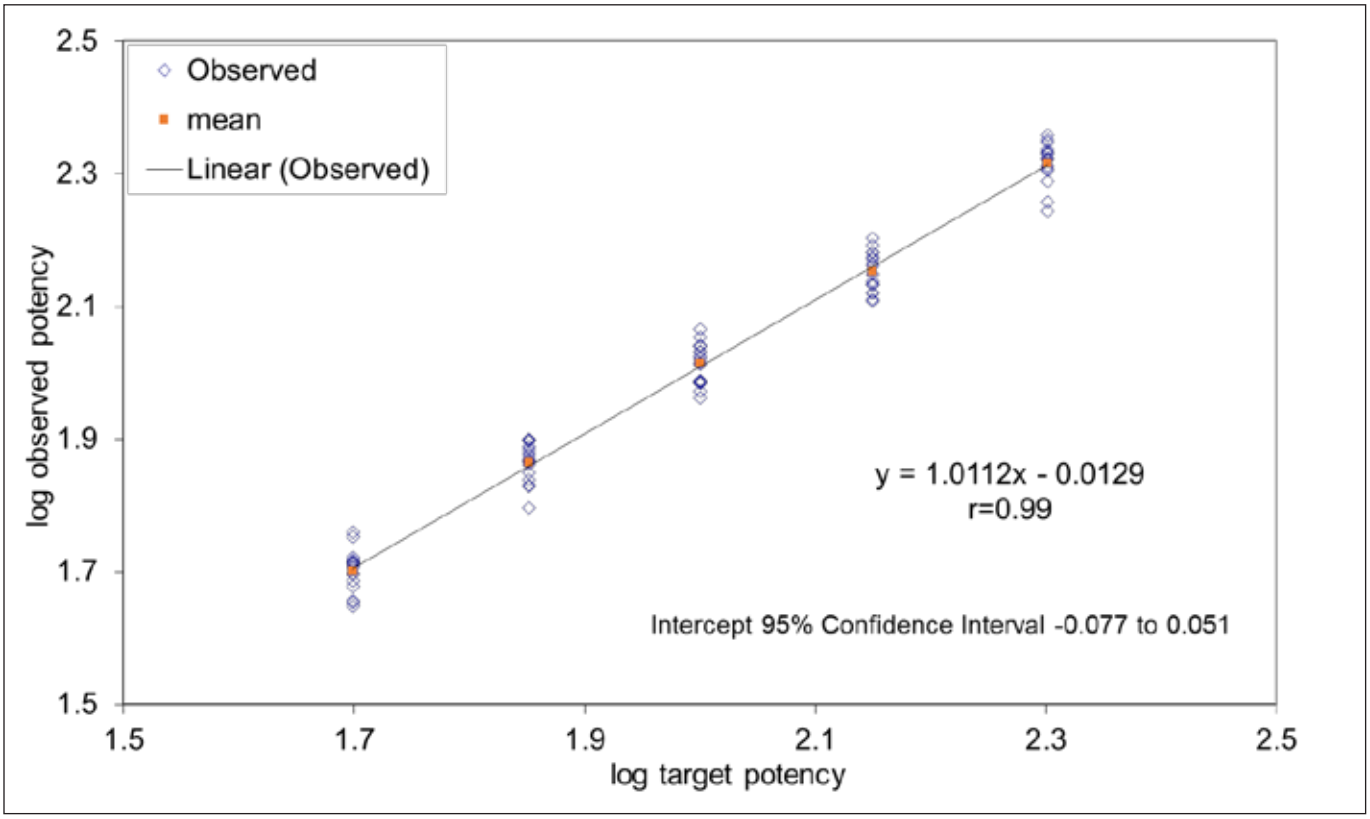
Target	Potency					Bias		
	Min	Max				Mean	Lower 90% CI	Upper 90% CI
50%	44.5	57.5	50.3	48.7	51.9	0.5	-2.6	3.8
71%	62.5	79.3	73.1	71.0	75.3	3.0	0.0	6.1
100%	91.5	116.2	103.1	99.9	106.3	3.1	-0.1	6.3
141%	128.0	159.4	142.0	137.7	146.4	0.7	-2.3	3.9
200%	175.1	227.8	207.3	200.8	214.0	3.6	0.4	7.0

Figure 2: %Relative Bias plot of a cell based potency assay



For the assessment of linearity, target potency values (50%, 71%, 100%, 141% and 200%) and the observed relative potency values are plotted as Log10 transformed data (Figure 3) and regression analysis performed on the data. Log transformation gives points that are equally spaced such that high concentrations cannot dominate the analysis. For the qualification data, the correlation coefficient was 0.99, with a slope of 1.0112 (90% confidence interval = 0.979, 1.0430) and the intercept (-0.0129) was within the bounds of the 90% confidence interval of zero intercept (-0.077, 0.051). This data met the acceptance criteria for correlation coefficient of $r>0.95$ and slope between 0.9 and 1.1.

Figure 3: Dilutional Linearity of a cell based potency assay



Intermediate precision was calculated by variance component analysis by ANOVA (5). The variance component analysis of the centre point data is shown in Table 4. For analysis performed on the same day (repeatability), each operator conducted two assays using independent potency preparations (two reference standard and ten mock potency samples over two dilution blocks), using the same cells and culture media. The five mock potency samples were loaded on different plate locations over the two assays. The within run component of variability (Var(err)) includes variability due to the cells, sample preparation and plate location. For analysis performed on different days (intermediate precision) an operator conducted two assays using independent preparations (as above), using a different cell bank and cell preparation, different culture media and different plate locations. The between run component of variability (Var(run)) includes variability due to cell bank, cell preparation, operator, day, sample preparation and plate location. The intermediate precision was less than 8.5% over the five mock potency samples and met the acceptance criteria GCV% <15%.

Table 4: Variance component Estimates and intermediate precision

Variance Component	50%	71%	100%	141%	200%	Average
Within run (Var(error))	0.0009759	0.0009817	0.001228	0.000364	0.0011881	0.000947
Between run (Var(run))	0.0000389	-0.000139	-0.00031	0.000594	-0.000207	0.000316
Intermediate precision (CSV%)	7.6	7.5	8.4	7.4	8.3	8.5

Note: value of 0 was used for the negative Var(run) values in intermediate precision calculations (7)

The precision, relative bias/accuracy and linearity data demonstrate the capability of the method to determine potency values over the range from 50% to 200%.

The variance component estimates (Var(error) & Var(run)) can also be used to calculate the precision of different assay formats. For the methodology present in this report the sample preparation is loaded on different locations over three plates, the reportable value is the result from the single assay. As can be seen from Table 5 there is a potential improvement in precision from 8.5% to 6.0% by increasing replication from one to two independent preparations. A similar improvement in precision is obtained by two independent assay runs, and this is a more effective method of reducing variability and potential bias in the reportable value.

Table 5: Format variability of the methodology

Replicate	Run (Assay)		
	1	2	3
1	8.5	6.0	4.8
2	6.0	4.7	4.2

The variance component estimates can also be used to determine the critical fold difference, that is the magnitude of difference that can be distinguished between samples in the same run (assay) or in different assay runs as would occurring during stability testing. A summary of the critical fold calculation is provide in Table 6. Not surprisingly the discriminatory power of the method is improved by replication.

Table 6: Critical fold difference of the methodology

Replicate	Run (Assay)					
	Release			Stability		
	1	2	3	1	2	3
1	1.18	1.12	1.10	1.26	1.18	1.14
2	1.12	1.10	1.09	1.18	1.14	1.12

It is important to understand the specification that the methodology will support and the potential for an Out Of Specification (OOS) as a result of assay variability. There are a number of formulae that can be used to determine process capability and it is generally accepted that for critical quality attributes the process capability should be 1.33 or better ( ). The capability (Cpm) of the methodology can be determined against the proposed specification and the probability of OOS can be calculated using the formulae provided in USP<1033> ( ).

In the early stages of development only a small number of batches may have been manufactured and the lot-to-lot variability (product variance) is often not known. The single assay run methodology used to generate a reportable value is able to support a specification of 71% to 141% with a 0.09% probability of OOS (Table 7). As the drug progresses towards phase III and commercialisation several batches will be manufacture and it is not unreasonable for a process to achieve between 5% variance in the critical quality attributes. Incorporating process variability not surprisingly increases the probability of OOS. To mitigate the probability of an OOS the precision and bias of the methodology must improve and this can be achieved by independent replication (Table 5). Increasing from one to two assay runs to generate the reportable value improves precision with a concomitant decrease in bias, the Cpm increases and the probability of OOS decreases (Table 7). These calculations provide information as to the level of risk of failing a batch due to both product and assay variability.

Table 7: Cpm and %probability of OOS against specification 71% to 141%

Specification	%variability			Replication	Cpm	Prob(OOS) %
	Precision	Bias	Process			
71% - 141%	8.5	6.6	-	1	1.10	0.09
71% - 141%	8.5	6.6	5	1	1.00	0.28
71% - 141%	6.0	6.2	5	2	1.30	0.009
71% - 141%	4.8	4.8	5	3	1.57	0.000

DOE FOR QUALIFICATION AND VALIDATION

The variance component estimates of intermediate precision from the High and Low points of a six factor ¼-factorial DoE (n=16), with replication is shown in Table 8. For each of the five mock potency samples the within run (Var error) estimate and between run estimates are a similar order of magnitude to that observed for the centre point DoE qualification data (Table 4). The overall intermediate precision was 9.2% and the 90% confidence interval estimating bias was 5.2%.

Table 8: Variance component Estimates and intermediate precision from the ¼ factorial DoE data

Variance Component	50%	71%	100%	141%	200%	Average
Within run (Var(error))	0.001282	0.000999	0.0007	0.001323	0.000846	0.00103
Between run (Var(run))	-0.00039	0.0000349	0.000541	0.000487	0.000608	0.000418
Intermediate precision (CSV%)	8.6	7.7	8.5	10.3	9.2	9.2

Note: value of 0 was used for the negative Var(run) values in intermediate precision calculations (7)

It is important to obtain good estimates of precision and bias as these impact on the format variability of the assay, critical fold difference, Cpm and OOS calculations. As an exercise, the variance component and intermediate precision estimates were calculated from a subset of the High and Low points of the DoE.

Selection of the data was based on a six factor ⅛-factorial DoE (n=8). For the subset of data the within run component (Var(error)) and between run component (Var(run)) were comparable to that detailed in Table 8 and estimates of intermediate precision (Table 9) were similar to the larger data set. The 90% confidence interval estimating the bias was less than 8.2%.

Table 9: Estimates of Intermediate precision from the DoE and subsets of data

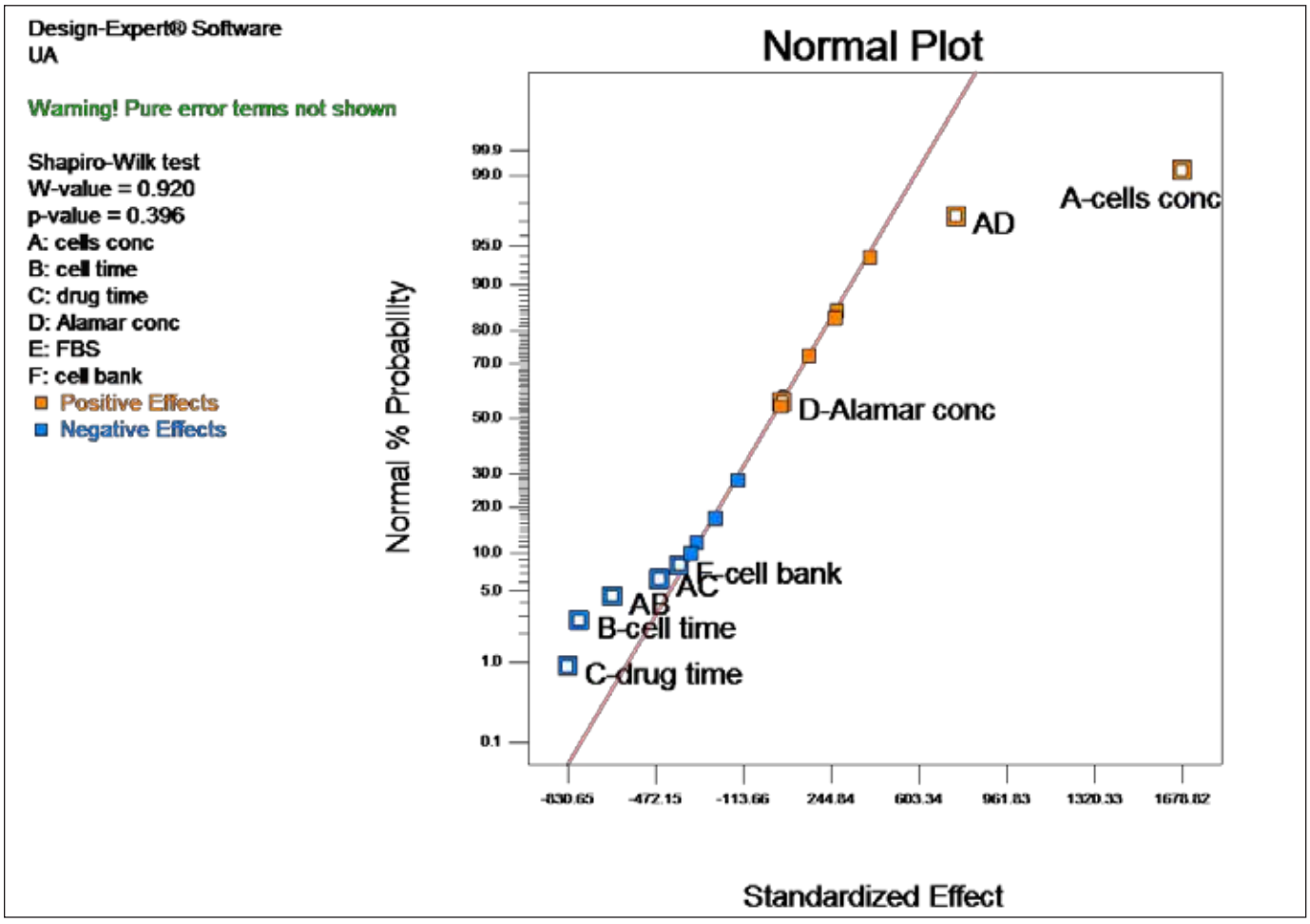
Number of runs	Intermediate precision (CSV%)					
	50%	71%	100%	141%	200%	Average
Qualification data (n=16)	7.6	7.5	8.4	7.4	8.3	8.5
Six factor DoE ¼-factorial (n=32)	8.6	7.7	8.5	10.3	9.2	9.2
Six factor DoE ⅛-factorial (n=16)	8.1	8.4	9.9	10.1	8.2	9.6

In the above studies mock potency samples were analysed as independent preparations in two assay runs providing replication at each event. The within run (repeatability) element ((Var(err)) was the largest component which increased as the sample size decreased. DoE is increasing being advocated for validation and a four factor DoE with replication would provide the number of assay runs to achieve good estimates of precision and bias.

DOE IN ASSESSING ASSAY ROBUSTNESS - SYSTEM SUITABILITY

Three plates failed system suitability and 2 individual samples at 200% failed due to poor replication / well to well variability. There were a number of factors that had either a positive effect or a negative effect on Alamar blue conversion (Figure 4), generating a range of values for the reference standard lower asymptote (437 to 1556), upper asymptote (1968 to 8116) and slope (0.77 to 1.14), but the absolute change did not impact on the similarity / parallelism criteria (delta lower asymptote, delta upper asymptote or delta slope) applied to the methodology. Cell concentration, not surprisingly, has a highly significant effect on the upper asymptote (Bonferroni correction) and to a lesser extent on the lower asymptote. Drug time and cell time exerted a negative effect on upper asymptote and lower asymptotes. There were no significant factors associated with slope.

Figure 4: Half-Normal plot showing factors having either a positive or negative effect on upper asymptote



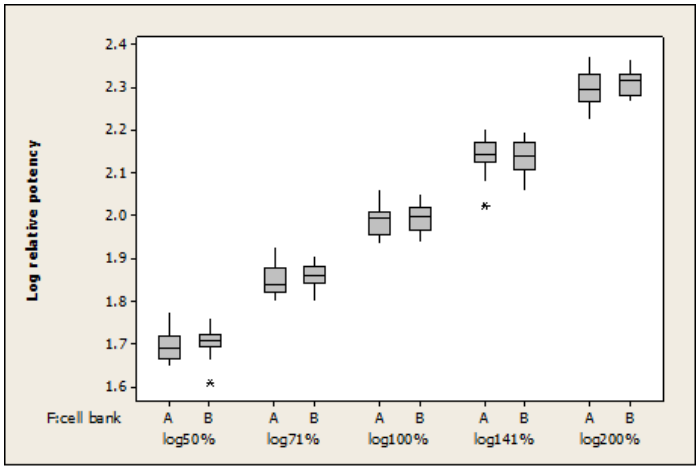
DOE IN ASSESSING ASSAY ROBUSTNESS – RELATIVE POTENCY

It is important to note that the critical fold difference (Table 5) of the single assay reportable value methodology was 1.18 for the repeated measurement of a single batch of material under the same conditions and 1.26 over different assay runs. For the centre point all values were within 118% of the nominal target. Similarly for the 3 hour and 5 hour High and Low point data all values were within 118% of the nominal target. Similar results were obtained for the 3-hour and 5-hour data and in a paired t-test there was no significant difference between the relative potency data (p= 0.37 to 0.96) for each of the five mock potency samples indicating that the assay is robust with regard to Alamar blue development time which was linear over time (not shown). The remaining discussion of the DoE data is with the 3 hour data, the 5 hour data generating comparable results. As there is considerable amount of data for brevity the following is provided as examples of the analysis.

The six factor ¼-factorial DoE with replication, centre point and additional assays, generated a total 60 independent assay analysed as pairs. The robustness of the methodology was assessed in Design Expert and Minitab software packages for changes in the mock potency reportable value, curve fit parameters and system suitability applied to the methodology.

In the DoE cell bank was a categorical variable and data was evaluated to determine if there was any significant difference between the two cell banks. An example of the relative potency data is provided in Figure 5, the Box plot showing overlap at each mock potency level between the two cell banks, and none of the pairings were statistically significant (p= 0.070 to 0.682). The Boxplot shows outliers (*) to that particular data set, which were not excluded in this or in subsequent analysis as these values represent variability in the methodology.

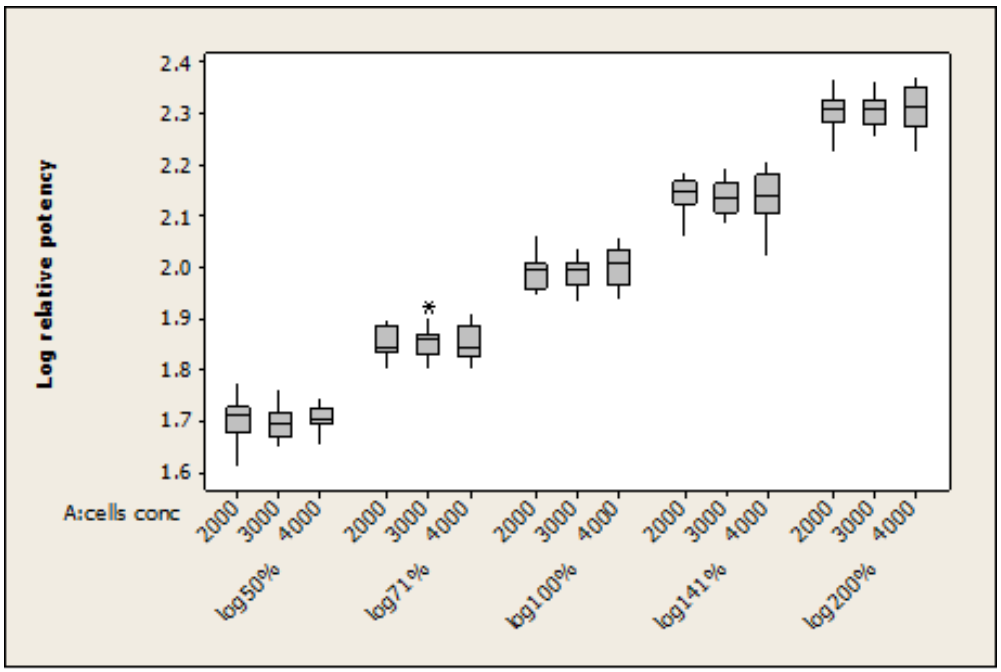
Figure 5: Boxplot of relative potency vs cell bank



When considered as an individual element, ie cell number as shown in Figure 6, there were no significant difference between the low, centre and high points of the DoE (ANOVA, p = 0.257 to 0.972) for each of the relative potency calculations, or for the curve fit parameters (upper asymptote, slope and lower asymptote) or system suitability parameters (reference standard EC50 and signal

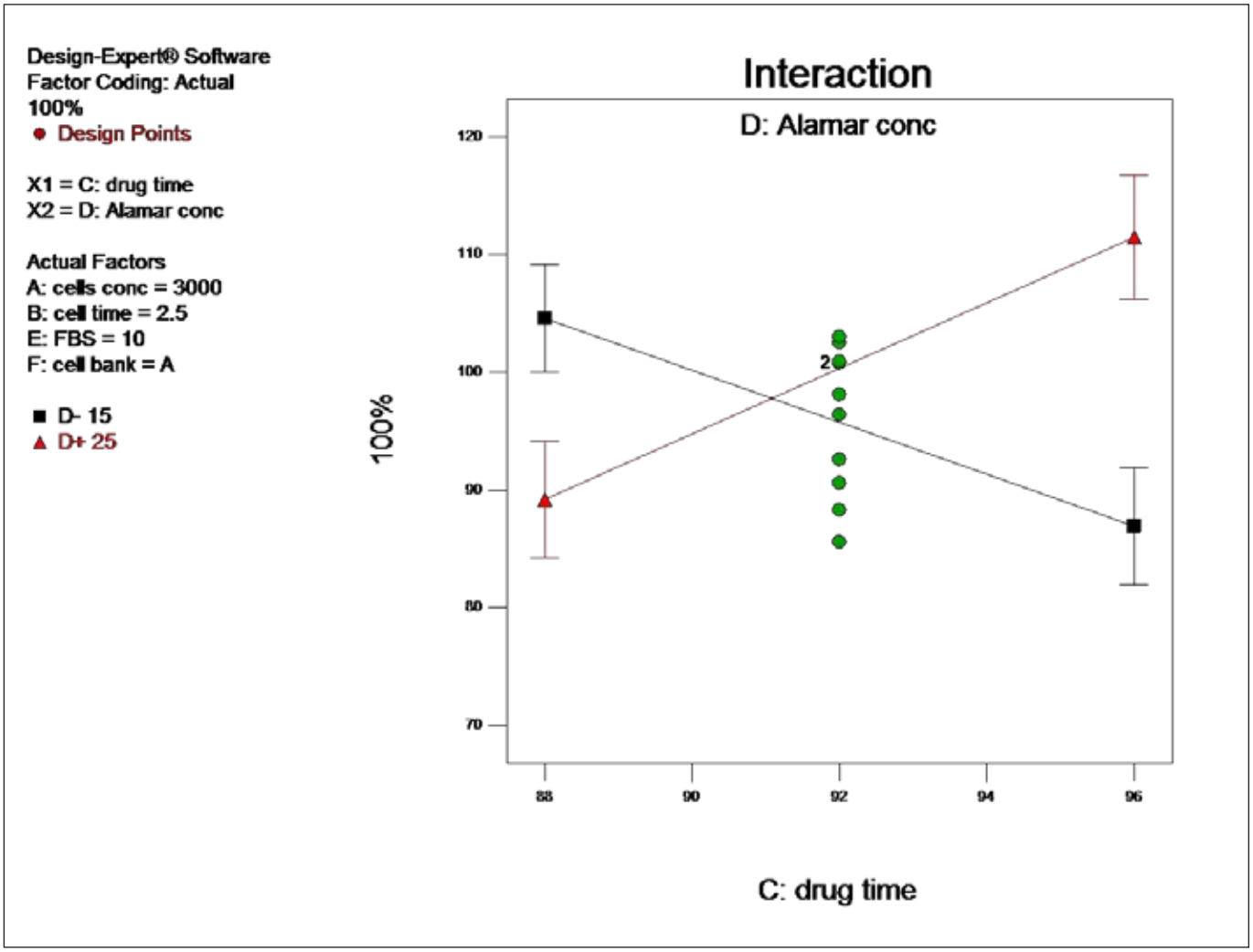
to noise). There was also no significant difference between operators (p= 0.061 to 0.593) and no significant difference between the 3-fold and 2.7-fold dilution series (p= 0.275 to-0.916).

Figure 6: Boxplot of relative potency vs cell number



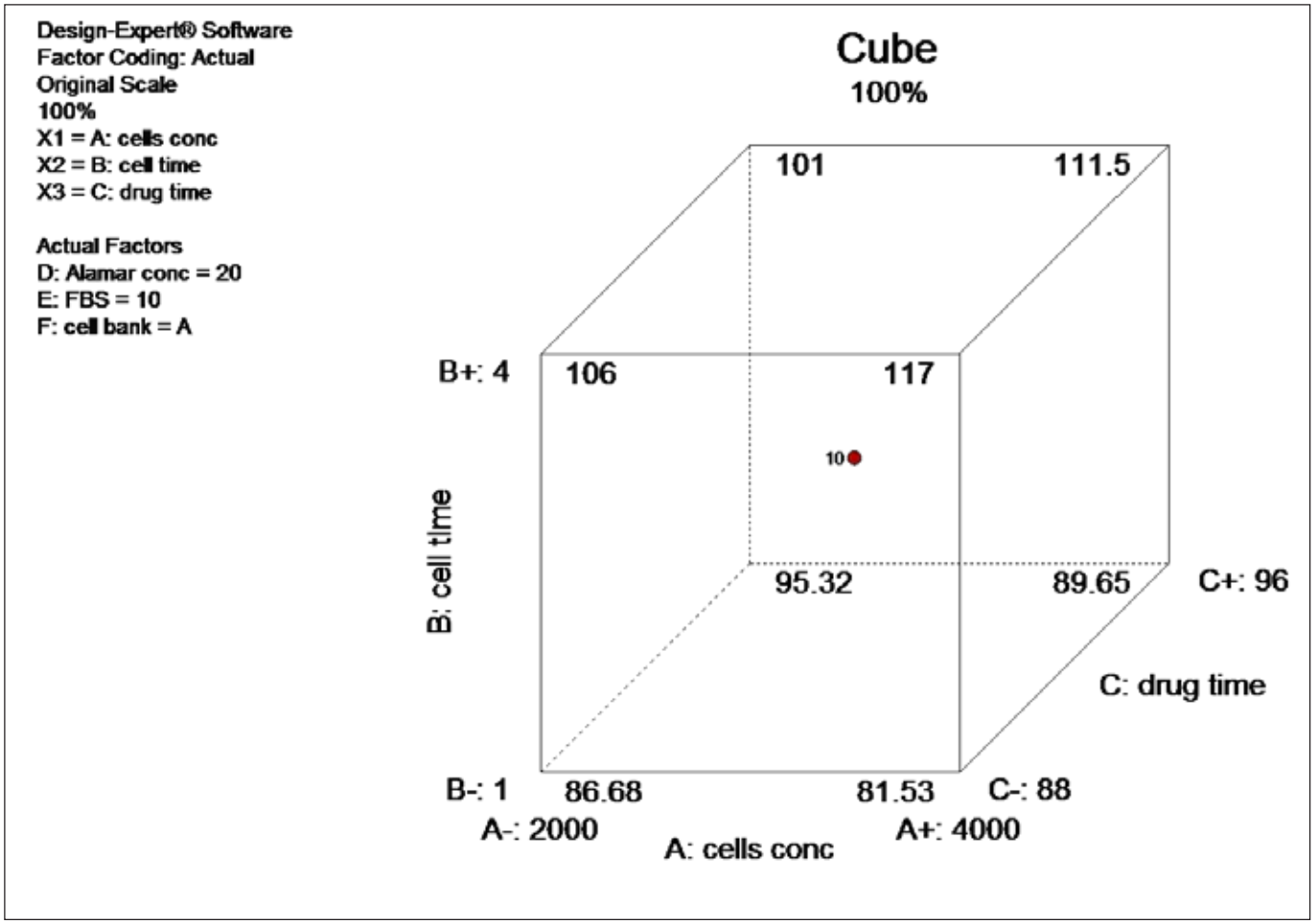
Design Expert Software was used to investigate possible interactions between the parameters and their effect on relative potency. Interpretation was difficult as there was no significant trend over the five mock potency levels, the data confounded by the resolution of the design and the number of factors selected in the models. Prior to model selection there was one significant interaction (Bonferroni correction) between drug exposure time and Alamar blue concentration for the 100%, nominal mock potency data, which was observed to varying degrees in the other four mock potency samples. This data indicated that those assays initiated using 15µL Alamar blue the relative potency decreased as drug exposure increased whereas those initiated with 25µL Alamar blue the relative potency increased as drug time increased (Figure 7). This interaction was confounded with other two factor and three factor interactions and some 40% of the model is due to lack of fit and pure error. Although some trends were evident in the 5 hour data there were no significant interactions (not shown) again highlighting the impact of individual sample variability on the model.

Figure 7: Interaction between drug time and Alamar blue concentration.



The design space for the 100% mock potency for three factors (cell number, cell time & drug time) with Alamar blue, %FBS and cell bank fixed is shown in Figure 8. This model indicates that with cell bank A there is an increase in potency as cell time increases but this effect was not observed for the model with cell bank B (not shown) and probably reflects the variability of the individual data used to construct the models.

Figure 8: Robustness of the method design space



Collectively the data indicates that methodology could be improved and to achieve this the assay components were moved to within the design space. The change in Alamar blue and %FBS concentration in the DoE was +25% and +20% of the nominal concentrations. It is highly unlikely that such a magnitude of change would occur during normal operation of the methodology and these parameters were fixed at the nominal concentrations. A single working cell bank is typically used for routine testing and new working cell banks are qualified for performance prior to use. For the assay factors, cell concentration was fixed to between 3000 and 4000 cells, cell recovery time was set to between 1 and 2.5 hours, drug exposure time reduced to 90 to 94 hours and Alamar blue time set to between 3.5 and 4.5 hours.

SUMMARY

The requirements for validation of bioassays, are defined in USP monograph <1033> (6) ICH Q2R1 (8) and the FDA and EMA Bioanalytical guidelines (9,10). The use of experimental design (DoE) is increasingly being utilised for bioassay validation and a multi-operator DoE is typically used to provide estimates of precision, accuracy/bias and to define the range of the methodology. This paper provides further evidence that qualification and validation can be achieved using DoE with similar precision and bias to that achieved with fixed parameters. The data demonstrated that the methodology was robust with regard to the assay parameters utilised, however these parameters were tightened to be within the "design space", and closer to that typically used during routine operation. The data also demonstrated that ruggedness factors such as cell bank, operator and reagent lot, factors that may change over the lifecycle, did not have a major impact on assay performance.

The data for the five mock potency samples were all within 118% of nominal potency with the centre point data generating similar precision (bias, range etc) to six factor 1/8- factorial DoE (8.5% vs 9.6%). In progressing towards commercialisation it is often necessary to combine several individual results (log transformed) to generate a reportable value within the GMP release specification. The data presented herein demonstrates that a single test result is able to support a specification 71% to 141%, however the capability index Cpm is 1.00. In progressing towards commercialisation the expectation is that Cpm should be greater than or equal to 1.33 ( ) and this can be achieved through replication.

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Ethical conduct

The studies used human cell lines, did not involve human subjects and did not require ethical approval.

Financial and competing interest's disclosure

The data presented is from an original assay conducted at Piramal paid by an external client. The studies were conducted using concepts detailed in USP monographs and the client gave permission to create a data set so that examples could be presented in this article. No financial conflicts exist.

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