Qualification of a mRNA-Based Potency Assay for GMP-Compliant mRNA efficacy Testing (Case Study)

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INTRODUCTION

Cell-based potency assays rely on living cells, involve multiple pipetting steps, and require the handling of small volumes - all factors that contribute to the inherent variability within assays, between analysts, and across different days. To mitigate these challenges, we implemented semi-automation using instruments such as the Integra VIAFLO96 and Integra ASSIST PLUS.

In our case study, we developed a potency expression assay in which mRNA is transfected into a suitable host cell line, and intracellular protein expression is quantified via ELISA. Following method optimization and the integration of semi-automation - necessitated by the complexity and the number of procedural steps - the method had to be qualified in accordance with the ICH Q2(R2) guideline.

RESULTS

Principle of the Cell-based mRNA Expression Assay

The method for determination of the relative mRNA expression was performed fully manually or semi-automated as below (Figure 1):

1. Integra ASSIST PLUS: selected cell-based assay (CBA) steps (blue).
2. Integra VIAFLO96: dilution and lysate transfer steps (orange).
3. BioTek 405TS: ELISA plate washing (green).
4. Absorbance measurement and PLA analysis

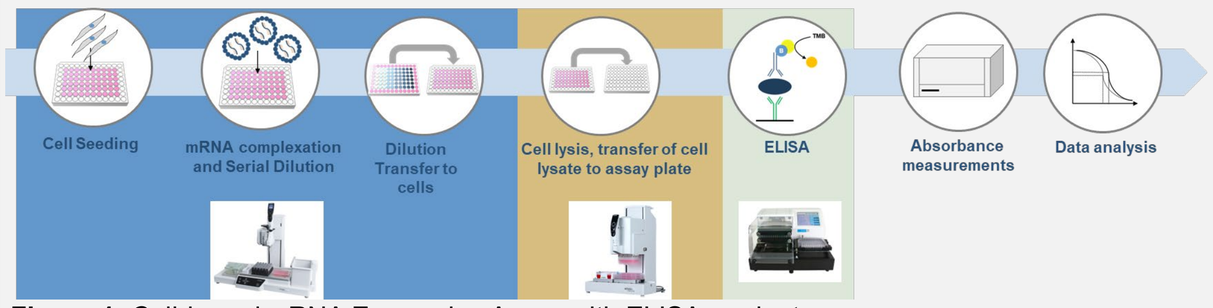

Figure 1: Cell-based mRNA Expression Assay with ELISA readout.

Method Qualification

Qualification parameters accuracy, precision (repeatability and intermediate precision), linearity, range and specificity/stability indication were assessed for a method range of 40-240% based on ICH Q2(R2):

- Nominal potency levels: 40%, 100%, and 240%
- Determinations: 4
- Variables: analysts, days, materials, instruments, semi-automation/manual
- Specificity/Stability indication (data not shown)

Representative dose response curves of the reference and the reference at different nominal potency levels are depicted in Figure 2 (no difference observed between semi-automation/manual) .

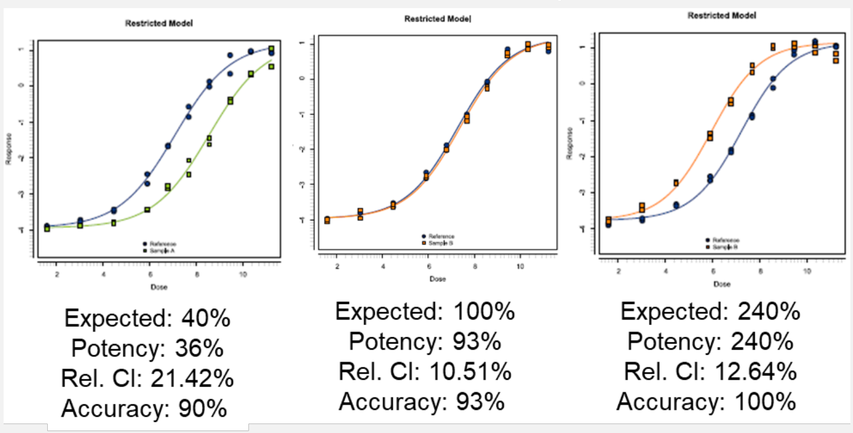

Figure 2: Representative dose response curve of the reference standard and reference material at 40% (left), 100% (middle) and 240% (right) expected potency.

Method Qualification - Accuracy

The accuracy of the method expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

- The reportable values ranged between 92-107%
- Mean Accuracy and Mean Bias were calculated (Table 1)

Table 1: Summary of accuracy results. n= number of results.

Potency Level [%]	n	Mean Accuracy [%]	Mean Bias [%]
40	4	95	7.8
100	4	101	4.4
240	4	98	4.6

The acceptance criteria for accuracy of 70-130% passed for each reportable value and the mean.

Method qualification - Repeatability

Repeatability of the assay expresses the precision under the same operating conditions over a short period of time. The CVg was found to be 5.6% (Table 2).

Table 2: Summary of repeatability results. n= number of results.

Potency level [%]	n	CVg [%]
100	4	5.6

The acceptance criterion for repeatability of CVg ≤ 20% passed.

Method Qualification – Intermediate Precision

The intermediate precision of the assay expresses within-laboratory variations of different days and/or different analysts. The CVg values ranged between 5.6 - 7.2% with an overall intermediate precision of 6.2% (Table 3).

Table 3: Summary of intermediate precision results.

Parameter	Potency level [%]			Mean
	40	100	240	
CV_g [%]	7.2	5.6	5.7	6.2

The acceptance criteria CVg ≤ 20% passed for each potency level and the mean.

Method Qualification – Linearity

The linearity of the assay expresses its ability (within a given range) to obtain test results which are directly proportional to the concentration of the test sample. Linearity was tested by regression analysis of assay results (observed potency) and expected potencies and revealed a Coefficient of Determination R^2 = 0.9943 (Figure 3).

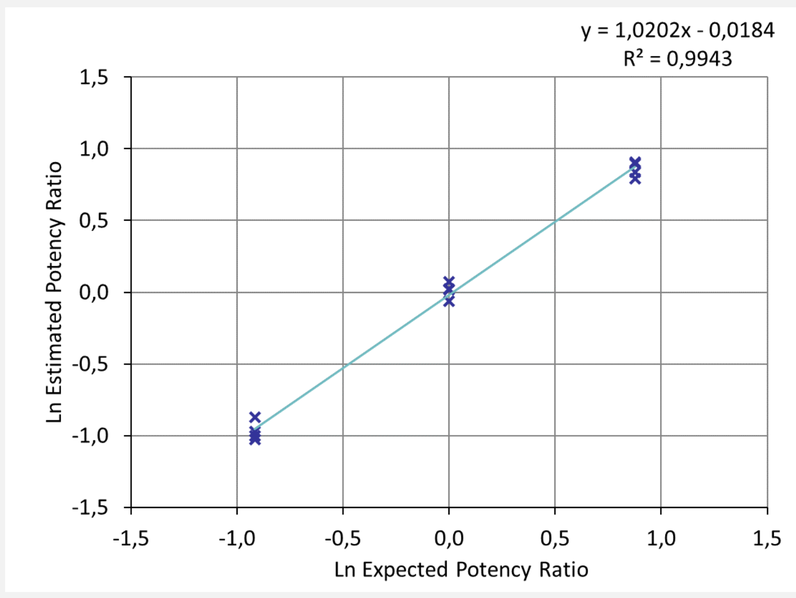

Figure 3: Regression line of ln(estimated potency ratio) versus ln(expected potency ratio) for the range from 40 % to 240 %.

The acceptance criteria for linearity $R^2 \geq 0.85$ passed.

Method qualification – Range

The acceptance criteria predefined for accuracy, precision and linearity passed, thus the range of the method was set to 40-240%.

CONCLUSION

We developed a cell-based mRNA transfection assay with protein quantification via ELISA readout for an mRNA therapeutic. To improve consistency and throughput, particularly in view of the large number of pipetting steps involved, we implemented semi-automation. The method was qualified according to ICH Q2(R2) guidelines and met the predefined accuracy, precision, linearity, range and specificity criteria across 40–240%. This case study demonstrates that semi-automation can enhance efficiency and reduce the manual workload.