Validation of a Flow Cytometry-Based Potency Assay for GMP-Compliant Antibody Testing (Case Study)

Learn more at www.eurofins.de/bpt/cpa

Dr. Serena Cuboni, Dr. Frances Reichert, Dr. Anika Anker and Dr. Alexander Knorre
Eurofins BioPharma Product Testing Munich GmbH, Planegg / Munich, Germany

INTRODUCTION

Flow cytometry is a powerful analytical technique which uses laser-based detection to characterise cellular properties, as well as to evaluate the binding and functional activity of therapeutic antibodies. One of the key advantages of this method is its ability to assess target antigens in their native conformation on the cell surface.

This case study outlines our validation strategy and results, offering practical insights into developing robust flow cytometry assays for reliable, regulatory-compliant biologics testing.

RESULTS

Principle of the Flow Cytometry Method

An indirect flow cytometry assay was established and optimized for antibody binding towards his target antigen (Figure 1).

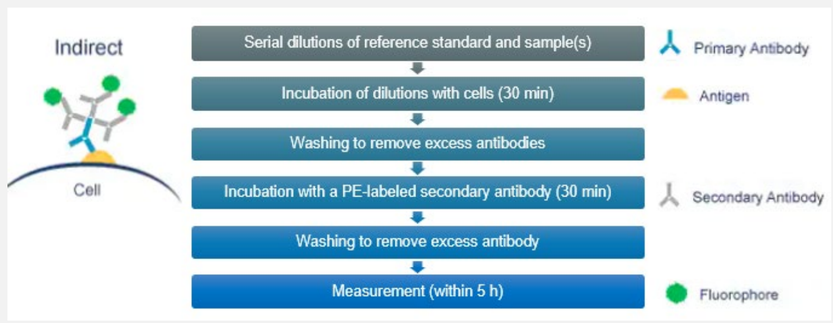

Figure 1: Indirect flow cytometry method.

Method Validation

Validation parameters accuracy, precision (repeatability and intermediate precision), linearity, range and specificity/stability indication were assessed for a method range of 50-200% acc. to ICH Q2(R1):

- Nominal potency levels: 50%, 71%, 100%, 141% and 200%
- Determinations: 6 (12 for repeatability)
- Variables: analysts, days, materials, instruments
- 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.

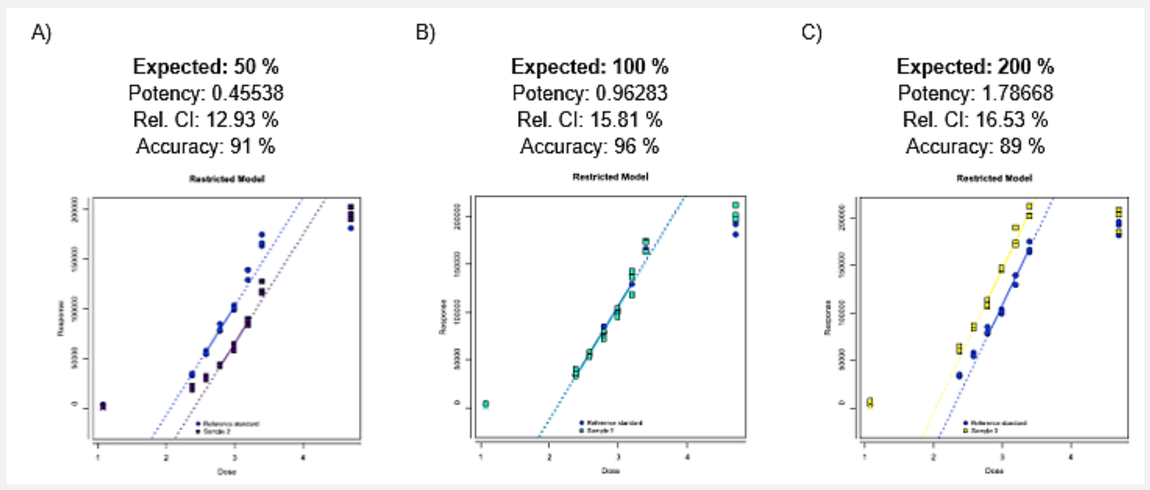

Figure 2: Representative dose response curve of the reference standard and reference standard at 50% (A), 100% (B) and 200% (C) expected potency.

Method Validation - 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 85-115%
- 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 [%]
50	6	100	8
71	6	99	9
100	6	99	5
141	6	93	8
200	6	100	5

The acceptance criteria for accuracy of 80-120% passed for each reportable value and the mean.

Method Validation - 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 5.7% (Table 2).

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

Potency level [%]	n	CVg [%]
100	12	5.7

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

Method Validation – Intermediate Precision

The intermediate precision of the assay expresses within-laboratory variations of different days and/or different analysts. The CVg values ranged between 5.9-11.7% with an overall intermediate precision of 8.6% (Table 3).

Table 3: Summary of intermediate precision results.

Parameter	Potency level [%]					Mean
	50	71	100	141	200	
CV_g [%]	9.4	11.7	7.6	5.9	7.3	8.6

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

Method Validation – 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 = 0.973 (Figure 3A) with equal distribution of residuals (Figure 3B).

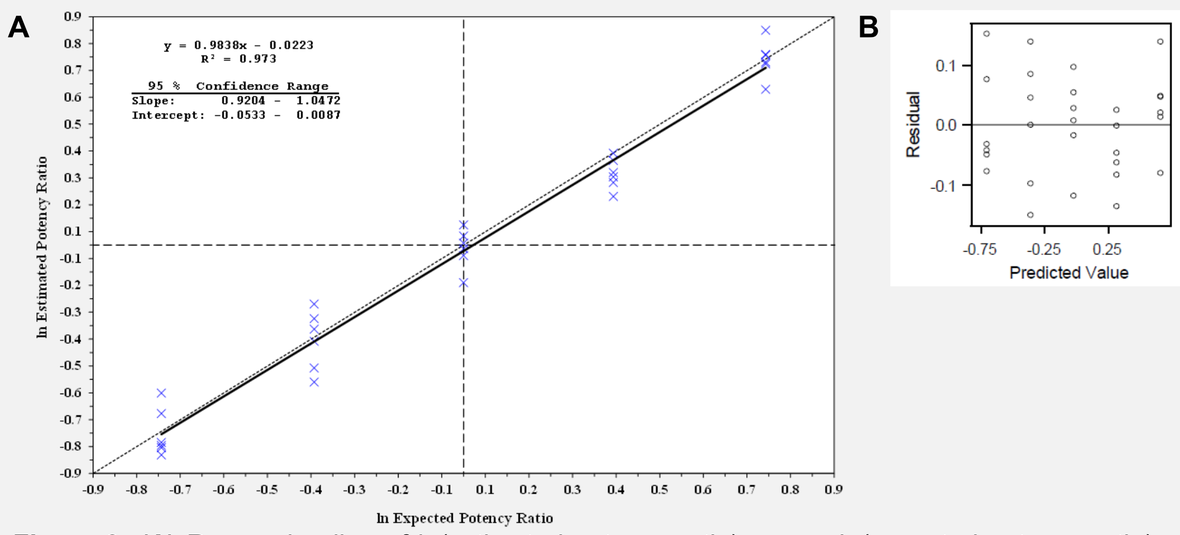

Figure 3: **(A)** Regression line of ln(estimated potency ratio) versus ln(expected potency ratio) (ln theor. PR) for the range from 50 % to 200 %. **(B)** Residual plot

The acceptance criteria for linearity R^2 ≥ 0.90 passed.

Method Validation – Range

The acceptance criteria predefined for accuracy, precision and linearity passed, thus the range of the method was set to 50-200%.

CONCLUSION

To support the quality control of a therapeutic monoclonal antibody for stability and release testing, we developed a flow cytometry-based potency assay to quantify the binding of this antibody to its membrane-expressed target molecule. Subsequently, this method was validated in accordance with the ICH Q2(R1) guideline, demonstrating fulfilment of predefined method acceptance criteria accuracy, repeatability, intermediate precision, linearity, specificity and stability-indicating properties for a method range of 50-200%.