From Validation to Bridging: A Case Study on Flow Cytometry Potency Assays

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INTRODUCTION

Antibodies consist of two structural regions: a variable fragment (Fab) that mediates antigen binding and a constant fragment (Fc) that mediates downstream effector functions. Flow cytometry, leveraging laser technology, is a powerful tool for analyzing not only cellular characteristics but also the binding of therapeutic antibodies towards their respective targets, with the distinct advantage of expressing the target antigen in its native conformation.

This case study discusses the validation results and instrument bridging data, offering insights into the key considerations, challenges, and best practices for flow cytometry-based assays.

RESULTS

Principle of the flow cytometry potency assay

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

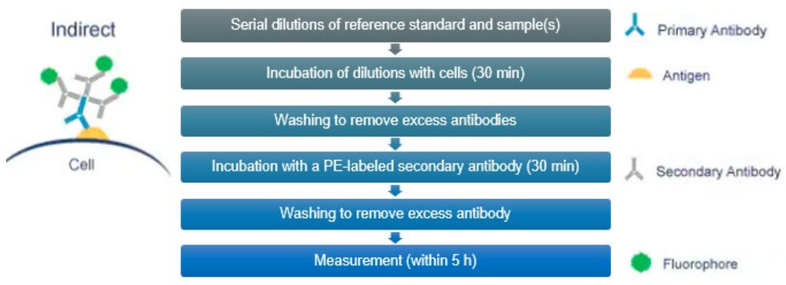

Figure 1: Indirect flow cytometry method.

Initial validation (instrument 1)

ICH Q2(R1) validation parameters were assessed for a method range of 50 - 200% (Table 1). Representative dose response curves are depicted in Figure 2.

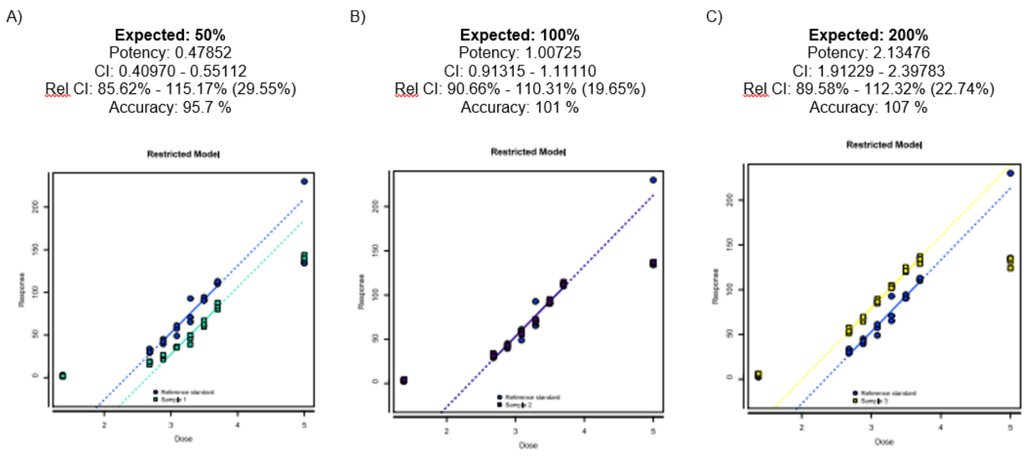

Figure 2: Representative dose response curve of the reference standard and reference standard at 50% (A), 100% (B) and 200% (C) expected potency using flow cytometer of vendor 1.

Method bridging challenges (instrument 2 & 3)

The validated method had to be migrated to another flow cytometer due to decommissioning of this initially used instrument.

Instrument 2 + 3:

Issues: SST and validation criteria failures (Figure 4)

Efforts: change of plastic materials, washing cycles, data acquisition and gating strategy

Outcome: fail

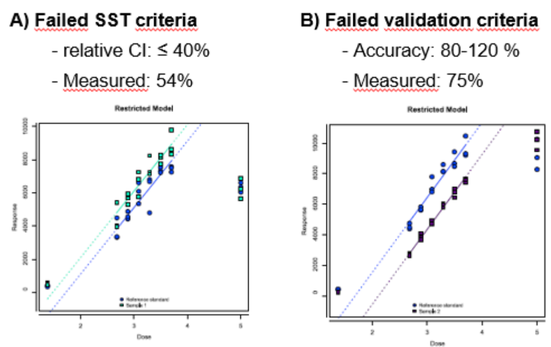

Figure 4: Results of failed SST criteria (A) and accuracy values (B).

Presumed root cause:

An increased instrument sensitivity compared to instruments of former generations in combination with the clustered antigen on the cell surface.

Bridging (instrument 3)

Due to failure in the method set-up using instrument 2 + 3, the following major method changes were required:

1. Decreasing the product concentration range
2. Decreasing the secondary antibody concentration

ICH Q2(R1) validation parameters were assessed for a method range of 50 - 200% (Table 1). Representative dose response curves are depicted in Figure 3.

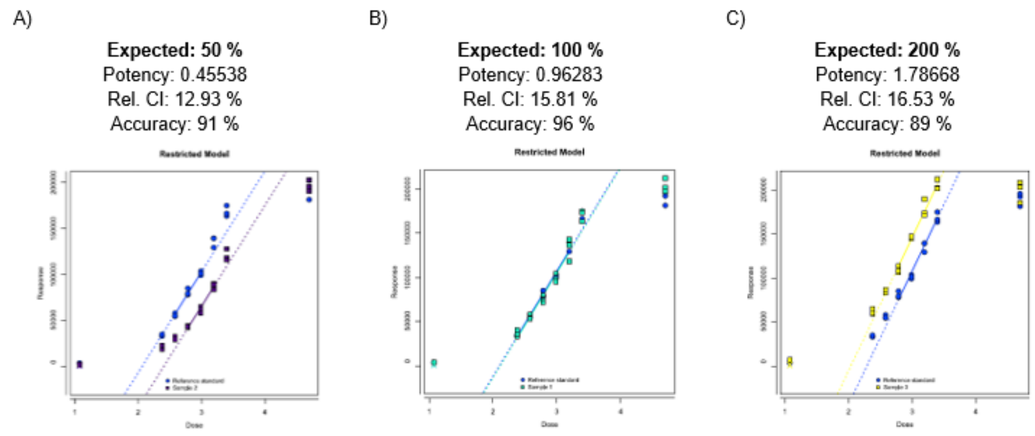

Figure 3: Representative dose response curve of the reference standard and reference standard at 50% (A), 100% (B) and 200% (C) expected potency using flow cytometer of vendor 3.

Validation and bridging results (instrument 1 & 3)

Results of the initial validation using instrument 1 and of the bridged, fully re-validated method using instrument 3 are summarized in Table 1.

Table 1: Results for initial validation using instrument 1 and for bridging using instrument 3.

Parameter	Acceptance Criterion	Validation (instrument 1)	Bridging (instrument 3)
Accuracy	80 to 120% for each sample	84 % - 116 %	93 % - 100 %
Repeatability	CVg ≤ 20%	4.4 %	5.7 %
Intermediate Precision	CVg ≤ 20%	2.4 % – 8.2 %, Mean: 6.0 %	5.9 % – 11.7 %, Mean: 8.6 %
Linearity	$R^2 \geq 0.90$	0.9885	0.9730
Range	accuracy, precision, linearity pass	50% - 200%	50% - 200%
Specificity and stability-indication	Unrelated molecule, DS/DP Matrix & spike, representative DP, Heat stressed & spike	No similar responses, Spike recovery within range	No similar response, Spike recovery within range
Robustness	Valid results to be reported	SST pass, potency within range	SST pass, potency within range
Equivalency	Potency of stability sample and QC in defined range	NA	potency within range
	Validation assessment	**PASS**	**PASS**

CONCLUSION

An antibody binding assay was established using flow cytometry and validated for a range of 50-200% acc. to ICH Q2(R1). The method has proven to be accurate and precise. Regression analysis revealed a linear relationship. However, only after changing the concentration range of the product as well as the secondary antibody dilution, the method was transferred and re-validated on a different flow cytometer.

RECOMMENDATION

In the past >15 flow cytometry methods (e.g. mRNA transfection, apoptosis, binding, intracellular staining) were transferred from or to other instruments without any issues (i.e. SST or method change not required). This led to the conclusion that not only the clustered antigen, but also the higher instrument sensitivity played a role for the failed method migration attempts.