Case Study - Improving Potency Assay Performance Through Modular Semi-Automation

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INTRODUCTION

For quality control (QC) of biopharmaceuticals, cell-based assays (CBAs) are essential for measuring the potency of commercial batch release and stability samples. Unlike ELISA or SPR binding assays, CBAs inherently exhibit greater variability due to factors such as multiple pipetting steps increasing assay complexity, analyst experience, and the use of living cells.
While full automation has been increasingly adopted in recent years, it remains a significant challenge, particularly for QC release assays in a Good Manufacturing Practice (GMP) environment. To address this, we have adopted a modular approach to automation, recognising that potency assays can be broken down into distinct steps, thereby allowing selective automation of specific parts of the assay.
Here, we share our experience about semi-automated potency assays by implementing Integra pipetting systems to improve assay consistency, minimise variability, and reduce analyst hands-on time, ultimately improving the reliability and robustness of potency testing in a QC environment.

RESULTS

Principle of cAMP Hunter™ Tirzepatide Bioassay Kit

A bioassay for Tirzepatide was developed using cAMP Hunter™ Bioassay Kit from Eurofins DiscoverX (Fig. 1).

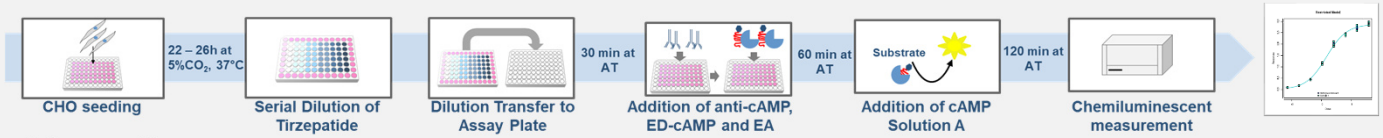


Figure 1: Bioassay workflow

Initial assay performance (manual)

Tirzepatide Bioassay was performed acc. to kit manual. Representative dose response curves are depicted in Fig. 2.

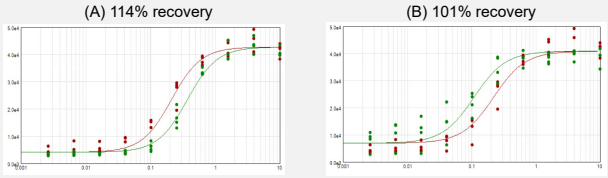


Figure 2: Representative dose response curve of the reference standard and reference standard at 50% (A) and 200% (B) expected potency performed manually.

Initial assay performance (automated)

Pipetting robot were purchased from INTEGRA Biosciences (model: ASSIST PLUS, Fig. 3) equipped with various volumetric pipettes. The system was categorized as class B as per USP <1058> and was released for GMP.

Figure 3: Integra ASSIST PLUS.

Initially, all method steps (Fig. 1) from cell seeding, dilution preparation, transfer, to detection reagent steps were programmed using Integra VIALAB software and transferred to the pipettes.

As depicted in Figure 4 semi-automation resulted in an increased replicate variability, significant curve shift and overestimated recovery for both potency levels 50% and 200%.

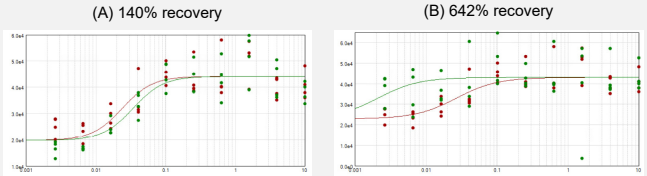
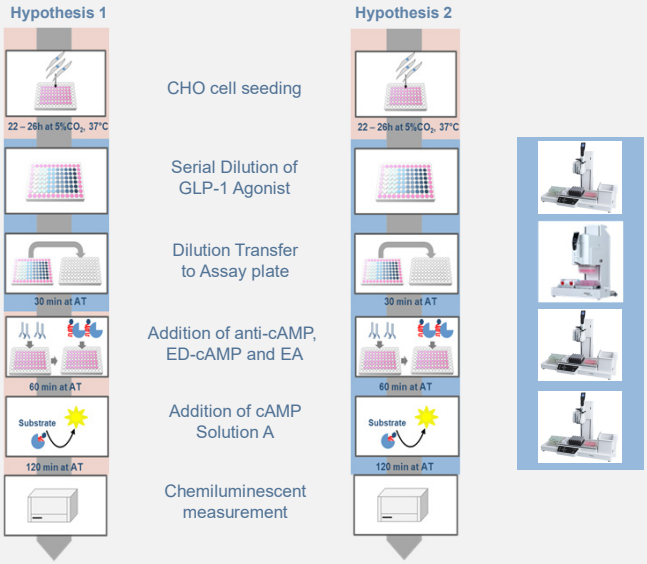


Figure 4: Representative dose response curve of the reference standard and reference standard at 50% (A) and 200% (B) expected potency performed semi-automated.

Hypothesis-driven optimization (automated)

Re-evaluation of the programming steps and pipetting times identified slow detection reagent addition as the most likely cause. To address this, two plates were run in parallel (manually (pink) and automated (blue)): (1) Hypothesis 1: 2-steps automated at a faster speed, while for Hypothesis 2: method was automated at a slower speed. While curve shape and variability improved, the results also revealed differences in assay windows (Figure 5).



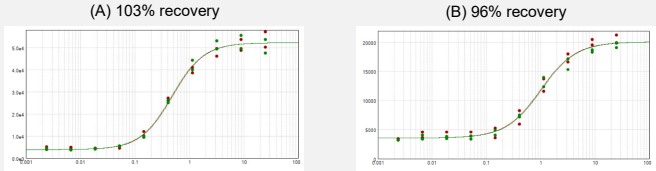


Figure 5: Dose response curve of the reference standard and reference standard at 100% expected potency performed (A) hypothesis 1 and (B) hypothesis 2. Pink: manual steps. Blue: automated steps.

CONCLUSION

The implementation of automation solutions such as INTEGRA ASSIST PLUS and INTEGRA VIAFLO96 enhanced the bioassay workflow. Initial results revealed significant curve shifts, increased variability, and overestimated recovery. By optimizing pipetting times and evaluating different pipetting procedures, we were able to improve the curve shape and reduce variability. However, differences in assay windows underscored the importance of careful optimization. Modular automation for QC potency assays not only improves assay consistency but also reduces analyst hands-on time, offering notable advantages over traditional single-channel or 8- and 12-channel pipettes.

RECOMMENDATION

Although automation enhances efficiency, careful optimization of all pipetting steps is the key to success.