Effects of Liquid Handling on Assay Performance

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Introduction

Scientists typically optimize assay parameters without consideration of liquid handler performance. Instead, default liquid handler values are selected during transition to an automated platform. This study illustrates the importance of optimizing both the assay and any associated liquid handling steps with the overall goal of minimizing variability.

Assay optimization parameters such as incubation time and temperature, and liquid handler optimization parameters such air gaps, aspirate/dispense speeds, fluid exit rates, and calibration were all studied. Parameters that bridged assay and liquid handling optimization such as mixing, buffer type, and labware were also considered.

The goal of this study was to optimize the entire assay and process prior to automation. By evaluating the entire assay assembly process, we aim to reduce variability as early as possible, thereby allowing assay transfer to become less troublesome. The study was designed to evaluate assay performance by comparing dose-response (IC_{50}), slope, curve span, and precision across the different parameters.

The model assay was based upon the binding protein streptavidin and its strong binding affinity toward biotin and derivatives of biotin. In this assay, biotin-4-fluorescein (B-Fl) competes with biotin and its derivatives for binding to streptavidin. If B-Fl binds, fluorescence is quenched. If it remains free in solution the fluorescence is maintained.

By altering various assay development parameters on a liquid handler, as well as varying parameters specific to automation, we demonstrated that some variations affected the outcome of the assay, while others did not. This methodology suggests that the user should pinpoint which variable liquid handler parameters have the most impact on the particular assay. Also highlighted is the importance of both developing the assay and qualifying the liquid handler for the assay in a concerted process optimization approach.

Methods

Experimental Set-up

1) Add 25 µL of 30 nM biotin-FL
2) Add 25 µL of buffer (or inhibitor)
3) Add 25 µL of 9 nM streptavidin (SA)
4) Incubate for 15 min (RT)
5) Read fluorescence (Ex 485 nm, Em 515 nm)

Two liquid handlers (LHs) were used to dispense each of the assay reagents:

- Thermo FinnPipette™ F2 12-channel manual pipette
- BioTek Precision™ XS automated liquid handler (ALH)

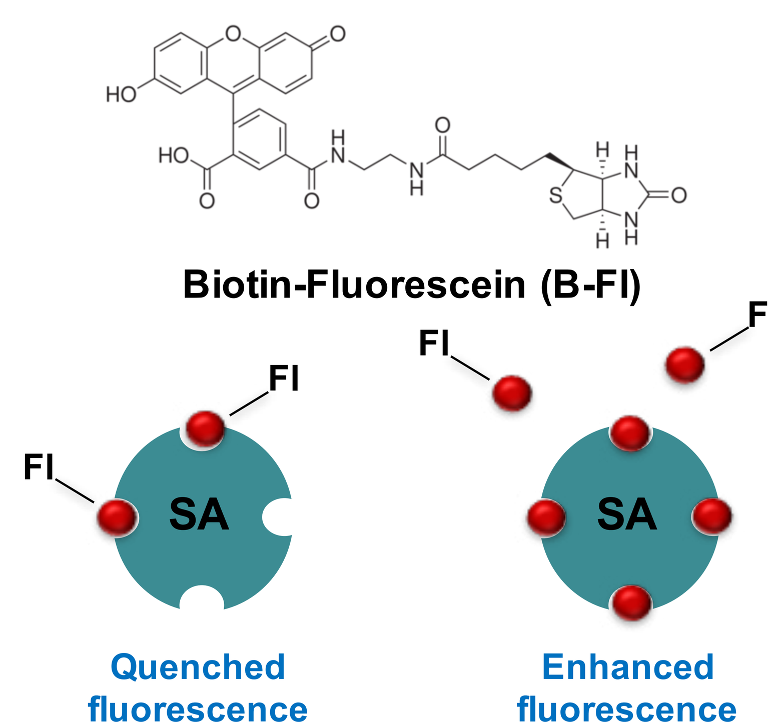

Figure 1: *Schematic of assay principle.* Upon binding of biotin-fluorescein (B-Fl) to streptavidin (SA), the fluorescence signal is quenched. A competitive inhibitor enhances fluorescence signal.

Parameter	Manual	Automated
Solution stability	✓	
Background buffer	✓	✓
Inhibitor	✓	✓
Inhibitor conc.	✓	✓
Incubation time	✓	✓
Mixing	✓	✓
Calibration		✓
Asp/Disp Rates		✓
Fluid exit rates		✓
Air gaps		✓
Plate type	✓	✓

Table 1: *Assay development parameters evaluated during this study.*

Project Phases:

Phase I: Develop and optimize the assay platform.

Phase II: : Evaluate effects of automated liquid handling parameters on the same assay.

Phase III: "Deconstruct" the assay: decide which parameters, when altered, result in significant variability.

Results

Assay Buffer

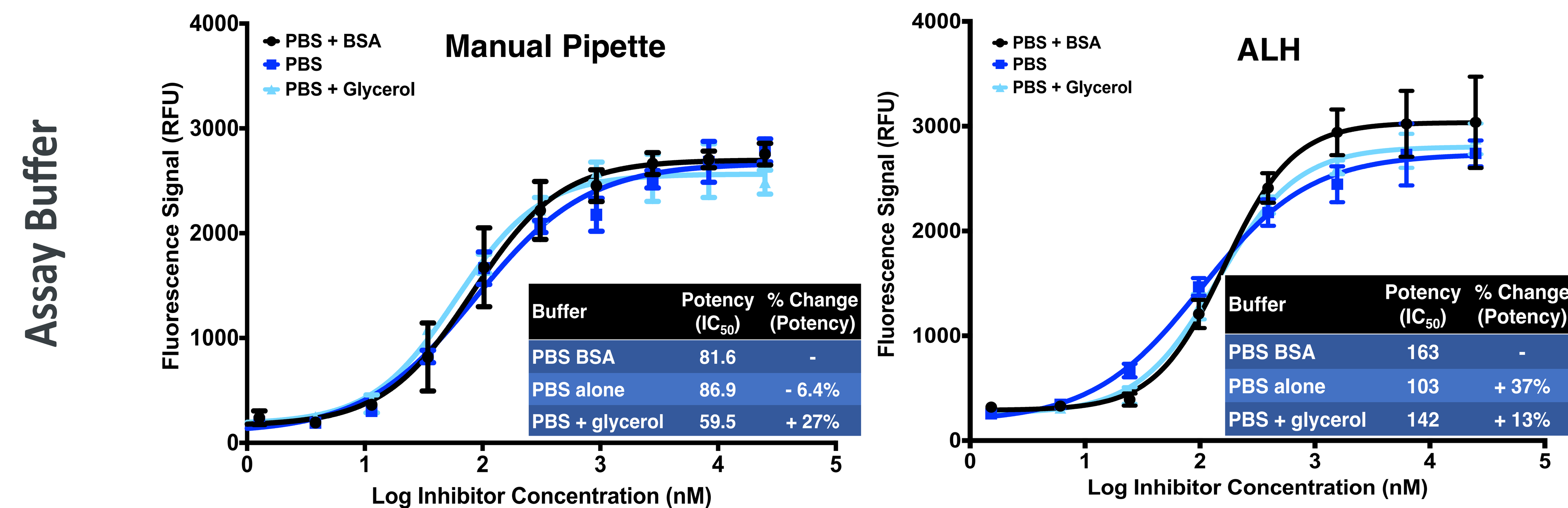

Figure 2: *Effects of assay buffer selection on reported inhibitor potency.* Assay was performed with either manual pipette (left) or ALH (right). Standard assay buffer is PBS BSA. When other buffers are used, the inhibitor potency reported by the assay is affected, however the effect varies depending on liquid handler.

Mixing

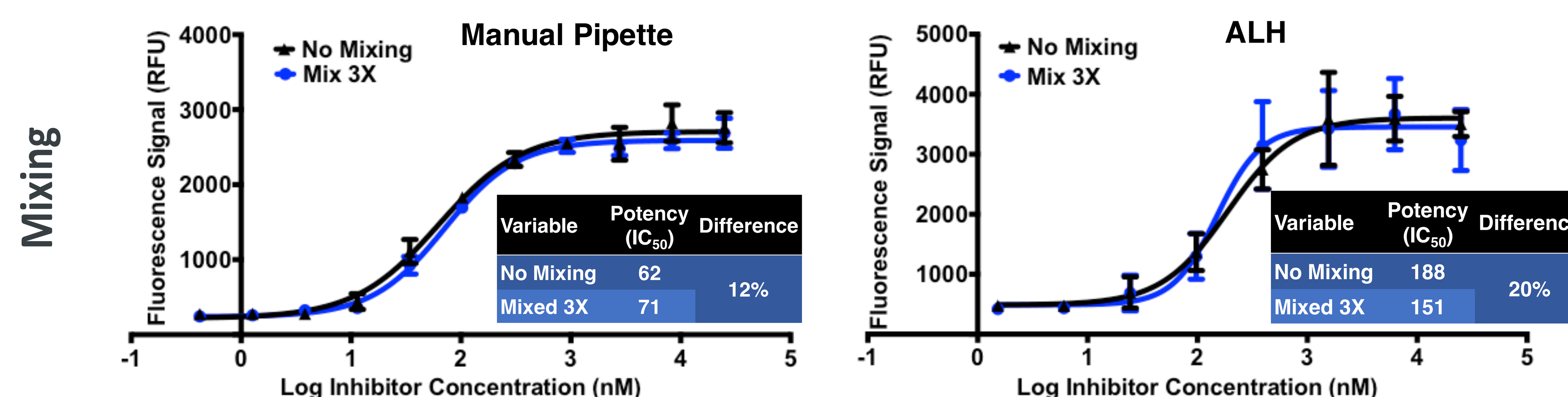

Figure 3: *Effects of mixing on reported inhibitor potency.* Assay was performed with either manual pipette (left) or ALH (right), and either not mixed or mixed three times during the final reagent addition. Effects on potency are evident with either liquid handling method, however the discrepancy is more pronounced on the ALH.

Reagent Stability

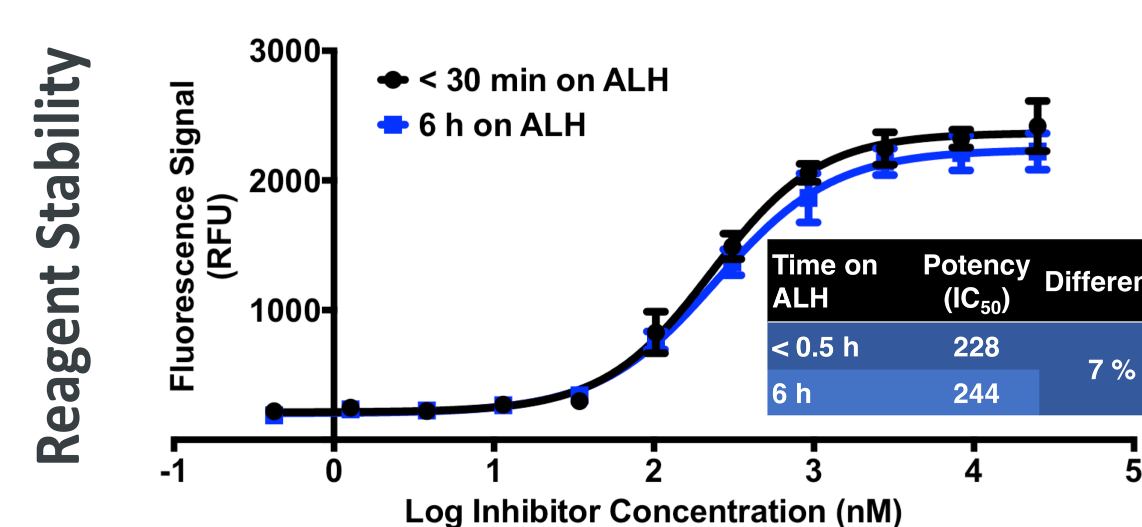

Figure 4: *Reagent stability on ALH affects assay outcome.* B-Fl, a bulk reagent for the assay, had limited stability at room temperature when left on the ALH. This represents an important consideration, specific to the ALH, if assay were to be run in a continuous, high-throughput manner.

Plate Type

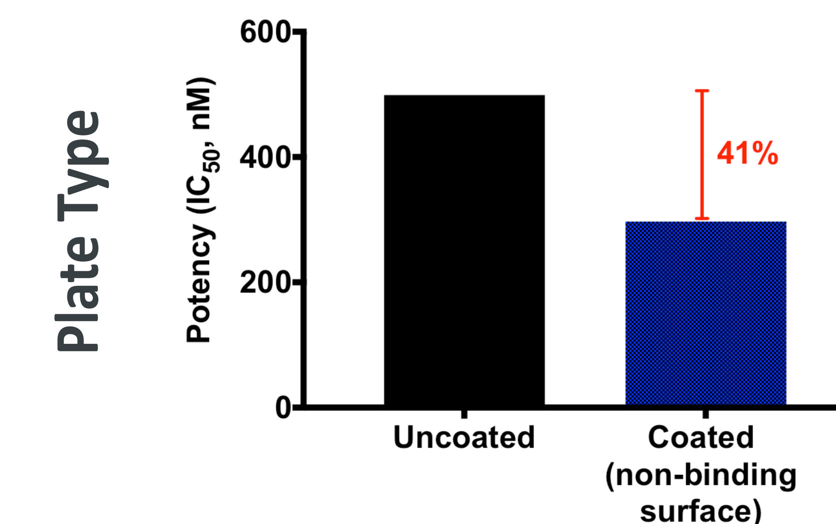

Figure 5: *Effect of plate type on assay outcome.* Consumables are often considered interchangeable. Two versions of the same source plate were used: uncoated and coated with an innocuous compound intended to decrease binding to plate surface.

Conclusions

- Assay optimization and ALH optimization must be performed together. Four assay parameters were identified in which optimization was needed during development and on the ALH: assay buffer selection, mixing, reagent stability, and plate type. Optimizing the assay alone, without considering the ALH, increases the risk of of additional error introduction resulting in prolonged/difficult method transfer.
- ALH parameters must be evaluated for each assay. Of the several liquid handling parameters available, three were found to be most important for this assay: improper ALH calibration, aspiration/ dispense rate and air gaps. Critical liquid handling parameters and potential sources of variability should be evaluated for each new assay.

Overall Impact

Optimizing the process as a whole (assay and ALH together) allows for better control to be gained over the process, thus reducing variability and minimizing assay transfer time.

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