A Holistic Approach to Assay Optimization for Automation

AUTHOR

Nathaniel G. Hentz, Ph.D.

NGH Scientific Consulting, LLC

E-mail: nghentz@gmail.com

Website: https://www.nghscicon.com/

ABSTRACT

This study illustrates the importance of optimizing both the assay and any associated liquid handling steps in parallel with the goal of minimizing overall assay variability. Assay optimization parameters such as incubation time and temperature, and liquid handler optimization parameters such pre-air gap volume, aspiration speed, and dispense speed were studied. Parameters that bridged assay and liquid handling optimization such as mixing, buffer type and labware were also considered. The aim of this study was to optimize not only the assay, but the process in its entirety prior to transfer to an automated liquid handling system. By evaluating the entire assay workflow, the goal was to understand sources of variability throughout the optimization process, thereby facilitating assay transfer. The study utilized a competitive binding assay as a model for evaluating assay performance by comparing dose-response/potency (IC_{50}), slope factor (also known as Hill slope), curve span, and assay variability across the different parameters. The model assay was based upon the binding protein streptavidin and its strong binding affinity toward biotin and its derivatives. In this assay, the fluorescent compound biotin-4-fluorescein (B-Fl) competes with a derivative of biotin, desthiobiotin (dt-Biotin), for binding to streptavidin. When B-Fl binds to streptavidin, fluorescence is quenched. In the presence of an inhibitor (e.g., dt-Biotin) fluorescence is restored when B-Fl is free in solution. By examining various liquid handling parameters, it was demonstrated that some affected the outcome of the assay, while others did not. This methodology allows one to focus on which liquid handler and assay process parameters could potentially have the most impact on a particular assay, thereby facilitating transfer.

KEYWORDS:

Liquid handling variability
Assay optimization
Assay performance
Automation
High-throughput screening
Quality

INTRODUCTION

Automation has been employed in drug discovery in order to achieve the level of throughput needed to test 10s to 100s of thousands of compounds in a relatively short period of time. Additionally, automation allows a higher level of precision than would otherwise be achieved manually. A common strategy for assay development is to first perform the assay manually. This may include mixing with handheld pipettes, evaluation of different buffer types, selection of appropriate labware, optimization of order of reagent addition, incubation times, determination of optimal reagent volumes, optimization of reagent concentrations, and designating equipment such as plate readers or automated liquid handlers. Once the appropriate assay specifications have been set, typically at bench-scale, the assay is then transferred to an automation platform or another laboratory. Assay transfers are typically further optimized by the development scientist, a combination of the development scientist and an automation engineer, an automation engineer. Two key areas that impact success of a high throughput screen are the assay quality and assay transfer.

Regarding assay quality, most high-throughput screening (HTS) groups include some sort of quality control program, whereby the primary focus is on ensuring that liquid handlers are functioning properly. By implementing a robust liquid handling quality control program, drug discovery laboratories can improve processes [1-8], improve quality of data [9], and thus minimize repeat testing or chasing false leads.

Of the major sources of assay variability (e.g., detection, liquid handling, biology, environment, and random error) that directly contribute to assay quality, drug discovery quality control programs tend to focus more on liquid handling [10,11]. In many cases, HTS labs rely on precision to assess liquid handling system performance. However, it has been shown that consideration of both accuracy and precision is necessary [12].

For a quality control program to be most effective, the entire process should be considered versus addressing individual components. In other words, it may be advantageous to study the entire assay workflow as a process within the context of the assay. For example, use of automated liquid handlers during screening campaigns includes initial compound preparation by dissolving a known amount of compound in a known amount of solvent, stock reagent solutions preparation, intermediate reagent dilution, and assay component addition (compounds, protein, substrate/ligand, cofactors, stop solution, etc.). At each step in these processes there is potential for volume error which can propagate into highly variable assay data. When all potential sources of variability are taken into consideration, the confirmation rate for compounds identified in HTS campaigns are typically 30-80% [13,14].

The other critical area is assay transfer. Any time an assay transitions from one drug discovery stage to the next (e.g., assay development to automation, HTS to lead optimization, lead optimization to SAR, etc.), an assay transfer takes place. Assay transfers are rarely seamless, and often result in long-lasting process delays due to troubleshooting, assay repeats, retraining, or re-validation. The problem can be exacerbated in the event of an automated liquid handler failure, when another system needs to replace the faulty one. In fact, assay (or method) transfers are an area of huge concern within the cGxP-regulated industry, as they are integral to the life cycle of any pharmaceutical product. As such, various regulatory guidances [15-18] and peer-reviewed papers [19-22] have been published to ensure success of assay transfers.

Of course, proper maintenance and calibration of liquid handlers is necessary for efficient assay transfer points or for replacing one liquid handler with another if failure occurs during a screening campaign. Unfortunately, calibrated instrumentation alone is not sufficient when it comes to assay transfer [22]. This study illustrates the criticality of evaluating various liquid handling parameters in conjunction with typical assay development variables, especially during the assay development/optimization phase. This study was designed to examine liquid handling performance and determine the effect of automated liquid handling parameters such as mixing, air gaps, aspirate rate, and dispense rate against different assay buffers. Specifically, a model protein binding (streptavidin) assay was used to demonstrate this principle. The effect of each parameter on the assay outcome was then measured via inhibitor potency (IC_{50}), slope factor, curve span and variability.

MATERIALS AND METHODS

Verification of Liquid Handler Performance

The Artel MVS (Nova Biomedical) was chosen to evaluate liquid handler performance because of the high degree of precision and accuracy required for this study. The verification method consisted of dispensing 10 µL, 20 µL or 50 µL of Reagent B Sample Solution (Nova Biomedical) into empty wells of a 96-well plate using a Agilent BioTek Precision XS microplate sample processor (Agilent Instruments) with accompanying 200 µL robotic pipette tips (Agilent Instruments) and a handheld 8-channel Finnpipette F2 (Thermo Scientific) with accompanying Finnpipette 200 µL disposable tips. Both the automated liquid handler and the handheld pipette were air displacement devices. Each well was then filled with Diluent Solution (Nova Biomedical) to a total volume of 200 µL. The plates were mixed for 1 min at 1500 rpm on a BioShake 3000 (Q.Instruments) prior to reading absorbance at 520 nm and 730 nm using the accompanying calibrated ELx800 NB plate reader (Nova Biomedical). The dispense volume was calculated using the dual-dye ratiometric methodology previously described [23].

Model Assay Principle

A model assay was developed at bench scale on a 96-well platform, where the effects of the various parameters on the assay outcome were studied. Applying this same principle to 384- or 1536-well format would also require consideration of well geometry. The model assay described in this paper was based on the tetravalent binding protein, streptavidin, and its affinity toward biotin and derivatives of biotin. In this assay [24], B-Fl (cat. no. B9431; MilliporeSigma) binds to streptavidin (from *Streptomyces avidinii*) (cat. no. S0677; MilliporeSigma) and upon binding, the fluorescence is quenched. In the presence of competitive inhibitors, B-Fl remains unbound and fluorescent. dt-Biotin (cat. no. D1411; MilliporeSigma) was used as the model inhibitor in this study because it has a similar dissociation constant as the B-Fl, both on the order of 10^{-10}-10^{-11} M [25,26]. The dt-Biotin stock was prepared as 5 mM compound dissolved in dimethyl sulfoxide (Fisher Scientific). Phosphate buffered saline (cat. no. P4417), bovine serum albumin (cat. no. A7906) and glycerol (cat. no. G5516) were from MilliporeSigma. The assay and all subsequent dilutions were carried out in phosphate buffered saline, pH 7.2, either as is or containing 0.1% bovine serum albumin or 0.1% glycerol. The basic assay scheme was:

1. Add 25 µL of B-Fl (labeled ligand).
2. Next, add 25 µL of dt-Biotin (inhibitor).
3. Finally, add 25 µL of streptavidin (binding protein).

The assay plates were shaken for 5 minutes then incubated at room temperature for an additional 25 minutes. The final concentrations of biotin-Fl and streptavidin in the assay were 30 nM and 7 nM, respectively. Fluorescence measurements were carried out on an Agilent BioTek Synergy 4 multimode plate reader (Agilent Instruments) with excitation at 485 nm and emission at 515 nm.

Optimization of this model assay was carried out by evaluating manual versus automated pipetting, effect of buffer, effect of various automated liquid handler parameters (e.g., air gaps, aspirate rate, dispense rate, and mixing), effect of reagent temperature, and effect of plate type. The magnitude of each effect was assessed by comparing changes in potency, assay variability, slope factor, and signal span.

Assay Optimization

Streptavidin and B-Fl assay concentrations were selected based on a previous report [12]. Buffer additives such as glycerol and bovine serum albumin were chosen to represent common solution types found in similar assays. All studies described in this work consisted of replicate 8-point inhibitor dilution curves (n = 6). Additionally, all reagents were prepared fresh at the time of each experiment in order to minimize variability due to stability. Prior to assay optimization experiments, the precision and accuracy were verified on both liquid handlers using their respective manufacturer recommended tips as previously described. The automated liquid handler and the handheld pipette were confirmed to deliver 25.0 µL ± 0.4%. Then a comparison was made between a handheld and automated liquid handler using the model assay. During this step, three assays were assembled, whereby one used phosphate buffered saline (PBS) as a diluent, the second used PBS + 0.1% bovine serum albumin (BSA), and the third consisted of PBS + 0.1% glycerol (GL). For each of these, the working solutions were prepared by diluting each reagent in the respective buffer. Labware was also investigated as part of the optimization. For this portion, black polystyrene, flat-bottomed 96-well plates (cat. no. 3915 and 3650; Corning) were compared to evaluate the effect of surface treatment (untreated versus non-binding, respectively). The untreated plate (#3915) was used throughout this study unless otherwise stated. The effect of reagent temperature was also investigated, whereby reagents were either fully equilibrated to room temperature (22 °C) or used immediately out of

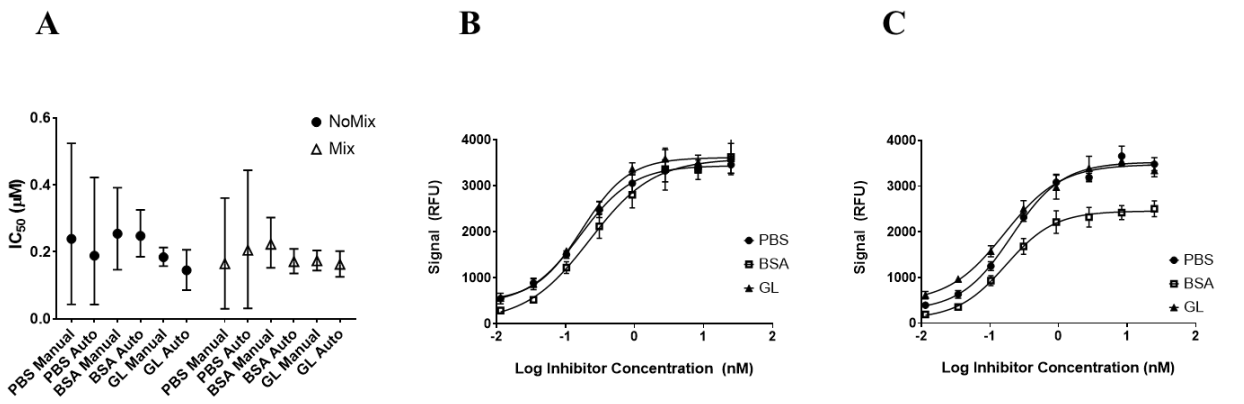

Figure 1. *Effect of mixing on assay variability (A) comparing no mixing versus mixing. "NoMix" indicates that the contents within each well were not intentionally mixed. "Mix" indicates that the contents of each well were mixed intentionally by repeated aspiration/dispense cycles (3x). Data points represent the average IC50 (n = 6) at a 95% confidence interval. "Manual" indicates mixing occurred by hand using a manual multi-channel pipette and "Auto" indicates the mixing by the automated pipettor. Effect of mixing on assay performance where the potency curves from manual (B) and automated liquid handler (C) mixing are compared with respect to each buffer type. Data points represent the average (n = 6) and the error bars represent ±1 standard deviation.*

refrigerated storage (4 °C). Finally, various automated liquid handler parameters were investigated: pre-aspirate air gaps, aspirate and dispense rates, and mixing. The default automated liquid handler parameters were set to 2 µL for the air gap and 2 for aspirate and dispense speeds unless otherwise noted. Also, the assay plates were covered with a plastic plate seal and allowed to mix on a plate shaker at room temperature for 5 ± 0.1 minutes, then incubated at room temperature (22 ± 0.5 °C) for 25 minutes (for a total incubation of 30 ± 0.5 minutes) unless otherwise stated. Since timing and temperature are critical for the competitive binding between B-Fl and dt-Biotin, particular attention was paid to these parameters. The streptavidin and B-Fl were prepared fresh, in bulk, and placed in a 50-mL reagent reservoir on the deck of the automated liquid handler, while the inhibitor, dt-Biotin, was prepared in a 96-well plate. Fresh tips were used after each dispense.

Potency Determinations

The inhibitor, dt-Biotin, was serially diluted 3-fold in replicate starting at 25 µM to yield 8-point dilution curves (n = 6 replicates per plate) and the reactions were monitored by fluorescence detection. The diluent was either PBS, BSA, or GL buffer. The signals (in RFU) were plotted against the log base 10 of inhibitor concentration and the resulting inhibition curves were analyzed using the "log(inhibitor) vs. response -- Variable slope (four parameters)" preloaded nonlinear regression model within Prism 7.04 (GraphPad Software), which is fit to "Y=Bottom + (Top-Bottom)/(1+10^((LogIC50-X)*HillSlope))". The resulting potencies (IC_{50}s) and assay variabilities were compared across the various liquid dispense parameters.

RESULTS AND DISCUSSION

Effect of Mixing on Assay Performance

To mimic manual assay development followed by transfer to an automated platform, mixing versus no mixing using both manual and automated techniques was compared. Additionally, the effect of buffer constituents on the mixing performance was determined. For both manual and automated dispense, the "no mix" plates simply contained reagents that were dispensed into the well followed by a 30-minute incubation at room temperature. Any mixing that occurred was induced by the action of adding fluid to each well and diffusion. For the "mix" plates, mixing was intentional and consisted of three cycles of aspiration and dispense upon addition of the final reagent. Again, the plates were then allowed to incubate at room temperature for 30 minutes.

As Figures 1A-C show, there were some differences in the assay results when manually dispensed reagents were compared to automated dispensing. Ideally, the assay should have performed the same (e.g., IC_{50}, assay span, slope factor, and variability) whether by manual or automated liquid dispensing or mixing versus no mixing. However, in the case of PBS as the assay buffer, there was a potency

Table 1. Effect of plate type on assay performance.

	Non-Binding Plate			
	PBS		GL	
Assay Parameter	**Manual**	**Auto**	**Manual**	**Auto**
Slope Factor	1.18	0.873	0.981	1.07
IC_{50} (µM)	0.222	0.227	0.214	0.241
Span	2080	1640	2530	1970
	Untreated Plate			
Slope Factor	1.14	1.17	1.28	1.06
IC_{50} (µM)	0.164	0.205	0.173	0.163
Span	3020	3250	3120	3020

difference of almost 21% when an automated liquid handler was used with no mixing (Figure 1A). A very similar trend was observed for the GL-based buffer, and no difference was observed with the BSA-based buffer (Figure 1A). Comparing the same conditions but with mixing after the final reagent addition revealed differences in transitioning from manual to automate dispensing (Figures 1B-C). In this case, both the PBS and BSA-based buffers yielded >20% difference in the potency response. The resulting potency from the GL-based buffer remained almost unchanged. Whether looking at mixing or no mixing, the slope factor showed differences between manual and automated pipetting. There was an 18% increase in slope factor when going from no mix to mixing for the PBS buffer. In contrast, there was no change for between no mix and mixing for the BSA and GL buffers. However, there was an almost 30% change when comparing manual mix to automated mix for these same buffers (Figures 1B-C). Additionally, BSA exhibited the largest change in assay span as can be seen in Figure 1C. These results were not entirely surprising since the mixing and diffusion are dependent on the fluid properties of the buffers used. This finding does demonstrate that disparities in behavior of various buffer types can occur when the assay is developed manually at first and then transitioned to an automated platform.

Assay variability (in the form of 95% confidence intervals) was also compared with respect to the buffer type and mixing. As seen in Figure 1A, while the overall assay variabilities are higher for PBS (0.210 ± 0.200 µM) than either BSA (0.224 ± 0.76 µM) or GL (0.174 ± 0.039 µM), the assay variability does not change significantly whether considering mix versus no mix or manual versus automated mixing. The assay variability using the BSA buffer, on the other hand, shows an improvement when going from manual no mix (0.254 ± 0.122 µM) to automated no mix (0.248 ± 0.70 µM) dispensing and no mixing manual (0.254 ± 0.122 µM) to mixing manual (0.223 ± 0.075 µM). By considering the GL buffer, Figure 1A shows an overall lowest assay variability (0.166 ± 0.039 µM), regardless of mixing versus no mixing or automated versus manual dispensing. It was observed that for dispenses not involving mixing, the PBS and BSA had a higher propensity for residual fluid remaining in the tips after dispensing. This is highly dependent on dispense rate, dry dispense versus wet dispense and air gap volumes and contributes greatly to variability.

Effect of Labware on Assay Performance

The effect of labware on assay performance was also evaluated by comparing two different assay plate types: Corning #3915 and #3650. Both plate types were black polystyrene, flat-bottom, 96-well formats used for fluorescence assays. The only difference is that one plate type was non-binding (#3650), while the other was untreated (#3915). As shown in Table I, whether manual or automated pipetting or considering buffer type, there was a consistent decrease in potency when using the non-binding plates. Also, the assay curve span was different as

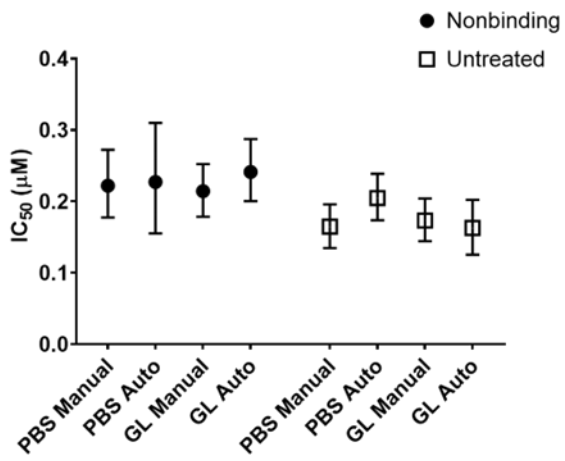

Figure 2. *Effect of plate type on assay variability. "Nonbinding" indicates the use of the Corning #3650 plate, while "Untreated" indicates the use of the Corning #3915 plate. Error bars represent the 95% confidence interval for replicate curves (n = 6).*

well, where a nearly 30% decrease was observed when using non-binding plates. This finding does not imply that one plate performs better than the other but instead stresses the importance of evaluating labware for its intended purpose.

During the investigation, a visual difference was observed in the liquid menisci once the assay was assembled in each plate type. To investigate the difference, both the untreated and nonbinding plates were used as *source plates* for the MVS reagent, then transferred into an Artel plate to evaluate volume accuracy and precision. After transferring MVS reagent from each assay plate type to a separate Artel verification plate, the solution from the non-binding plate clearly looked different in the destination plate compared to that of the untreated plate, thus indicating that the plate treatment had likely altered the fluid properties (data not shown). For example, the Artel plate containing solution dispensed from the non-binding source plate displays a more pronounced convex meniscus compared to a plate containing solution dispensed from the untreated source plate. The precision and accuracy as measured by the MVS system were markedly different for each source plate type (data not shown). This observation was similar to the performance of the assay in each plate type. As a result, only the untreated plate was used as a source plate for all subsequent experiments.

Overall, for this assay there did not appear to be a significant advantage in manual dispensing versus automated dispensing when using either the untreated or non-binding plates when considering only potency. However, there is an improvement in assay variability for PBS buffer when using the non-binding plate (0.227 ± 0.77 µM) compared to the untreated plate (0.205 ± 0.033 µM) (Figure 2).

During the assay optimization the effect of reagent temperature on dispense was also investigated by measuring assay performance. Reagent temperature appeared to affect the assay performance when comparing reagent dispenses at room temperature and chilled (~4°C). In this case, two parameters showed differences: a nearly 18% increase in slope factor was observed when pipetting at room temperature compared to cold; and the IC_{50} dropped by almost 32%, which correlates to a higher potency. An improvement in assay variability was observed when pipetting reagents that had fully equilibrated to room temperature (0.235 ± 0.125 µM) versus dispensing at ~4°C (0.311 ± 0.285 µM). This difference can be explained by the fact that since temperature is not necessarily controlled on the deck of the automated liquid handler, the cold reagents were warming unevenly across the plate during the liquid transfer. The temperature discrepancies then affected the volume aspirated and dispensed; a known phenomenon [27].

Effect of Automated Liquid Handler Variables on Assay Performance

Once the assay parameters were optimized (e.g., plate type, dispense temperature, and mixing), three automated liquid handling parameters were investigated: air gap, aspirate speed and dispense speed. To be clear, there were numerous automated liquid handling parameters that could have been

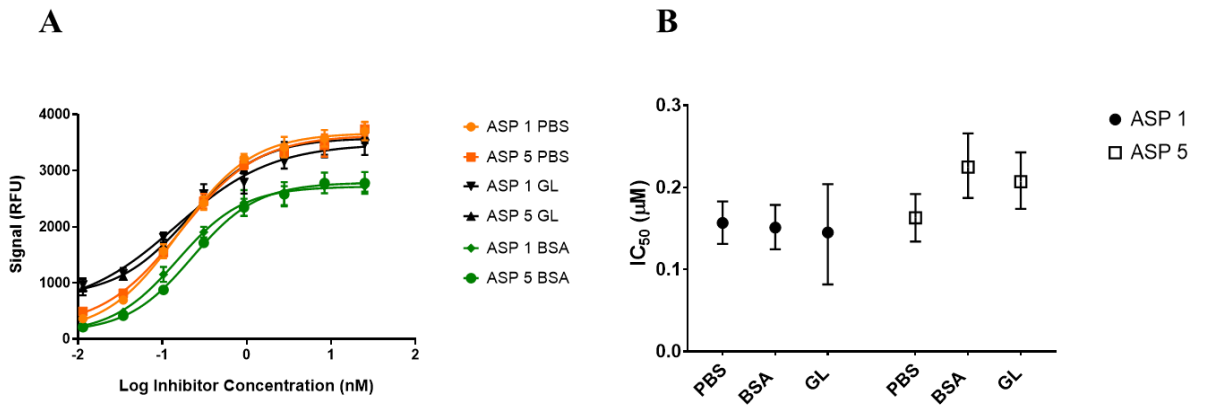

Figure 3. *Effect of aspirate (ASP) speed on assay performance (A) and variability (B). An aspirate speed of 1 is the slowest setting and 5 is the fastest. Error bars in the potency curves represent ±1 standard deviation, while those for the variability plot represent the 95% confidence interval. All data points in both plots represent averages (n = 6).*

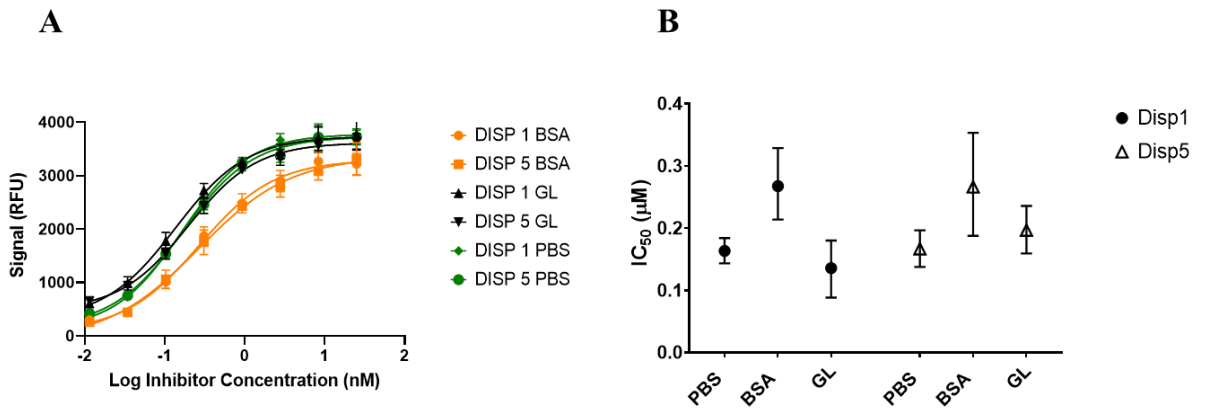

Figure 4. *Effect of dispense (DISP) speed on assay performance (A) and variability (B). A dispense speed of 1 is the slowest setting and 5 is the fastest. Error bars in the potency curves represent ±1 standard deviation, while those for the variability plot represent the 95% confidence interval. All data points in both plots represent averages (n = 6).*

evaluated, but these three parameters were selected because of the likelihood that they would have the biggest impact on this assay due to the solution viscosities and propensity for bubbling. Aspirate speeds of 1 (slowest) and 5 (fastest) were investigated against the three assay buffers. Both the slope factor and IC_{50} for the PBS buffer were unaffected by aspirate speed (Figure 3A). However, the IC_{50} for BSA buffer was affected (nearly a 30% decrease in potency when using the fastest setting), while the slope factor remained unchanged. When changing aspirate speed, the GL buffer demonstrated a larger effect. Both the slope factor and IC_{50} differed by nearly 30% for each. This is likely due to the combination of a change in surface tension and slight increase in solution viscosity for 0.1% glycerol as compared to PBS. It is known that temperature can affect the viscosity of aqueous based solutions to the same degree with only a 0.1°C change. However, in this case reactions with all three of the solutions either occurred on a single plate and experienced the same temperature fluctuations even if minor or were pipetted within a short period of time of one another if on separate plates.

By analyzing assay variability (Figure 3B), no significant change was observed between the slowest and fastest aspiration speed settings for the PBS buffer. Although, there appears to be a slight decrease in assay variability for GL by increasing

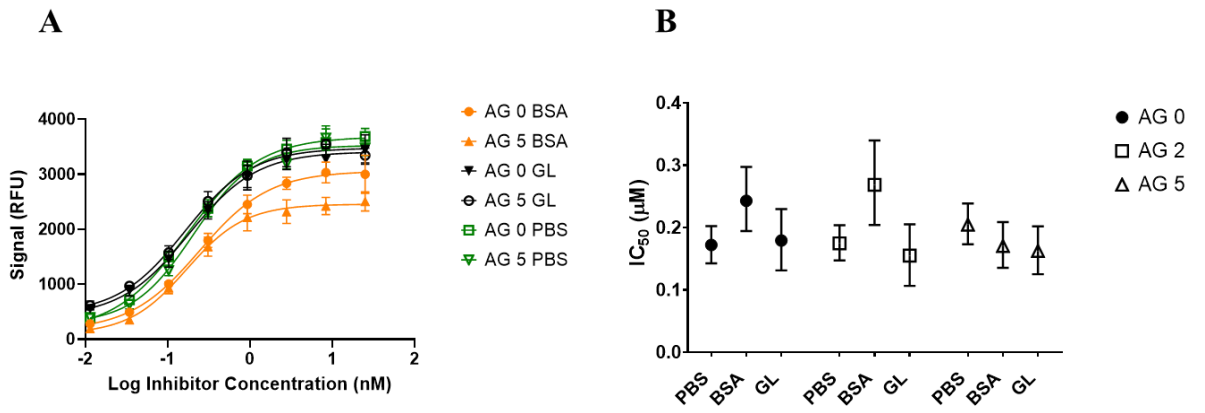

***Figure 5**. Effect of air gap (AG) on assay performance (A) and variability (B). An air gap (pre-air gap) of 0 indicates 0 µL, 2 indicates 2 µL, and 5 indicates 5 µL of air volume dispensed following the solution dispense into each well. Error bars in the potency curves represent ±1 standard deviation, while those for the variability plot represent the 95% confidence interval. All data points in both plots represent averages (n = 6).*

aspirate speed and a slight increase for the BSA buffer.

The dispense speed (1 being the slowest and 5 being the fastest) had a greater effect on the assay in the GL-based buffer, where the slope factor changed by 7% and the potency changed by about 30% (Figure 4A). Although there were differences in slope factors that corresponded to dispense rate, the potency for either PBS- or BSA-based assay buffers exhibited almost no change. The assay variability was not significantly affected for PBS- and GL-based buffers (Figure 4B). There was a slight worsening in assay variability when the BSA buffer was used, which correlated with an increased number of bubbles observed in the wells just prior to reading the plate (data not shown).

The final automated liquid handler parameter that was evaluated was pre-air gap, which is defined as the volume of air aspirated before the fluid. As displayed in Figure 5A, an air gap setting of 5 µL led to a decrease in potency (19%) for PBS as the assay buffer compared to a setting of 0 µL, while the slope factor increased by 18%. For the BSA assay buffer, an air gap setting of 5 µL yielded an increase in potency by 70% and a nearly 20% increase in slope factor. For comparison, little change was observed with the GL-based assay buffer, either in potency or slope factor.

As demonstrated in Figure 5B, there were multiple contributions to variability in this assay when examining the effect(s) of air gap volume. Assays run with PBS were unaffected by changes in air gap settings regardless of volume. Assays containing BSA exhibited the highest assay variability at an air gap volume of 2 µL (0.269 ± 0.068 µM), but the outcome was improved when air gap volume was changed to 5 µL (0.171 ± 0.037 µM). A slight improvement in assay variability was also observed in assays containing GL by using a 5 µL air gap volume (0.163 ± 0.038 µM) compared to a 2 µL air gap (0.155 ± 0.049 µM).

CONCLUSIONS

It has been reported previously that there are three best practices that ensure proper functionality of liquid handling, whether manual or automated: regular maintenance (e.g., cleaning, inspection and lubrication), performance checks (e.g., system suitability) and calibration. Also, it is recommended that the performance checking and calibration should investigate both precision (variability) *and* accuracy (delivered versus expected volume) of the delivered volumes. As critical as those procedures are, it takes more than that to minimize assay variability and thus improve data quality. In fact, including liquid handler optimization as part of the assay design process from the very beginning can improve the ease of process transfer and success of the assay at the onset. By taking this holistic approach to assay design and optimization, it is reasonable to expect method transfers to be more successful. This report demonstrates that the entire assay, including the intended final liquid handling process, must be considered during optimization. This extends into manual versus automated pipetting and various parameters within an automated liquid handling platform. This report even shows a difference in the behavior of the assay buffer in assay performance when transferring from manual to an automated liquid handling platform.

The findings in this paper have important implications. First, it was found that buffer type made an impact when the assay transitioned from manual pipetting to automated pipetting; both in terms of assay descriptors (e.g., potency, assay span and slope factor) and assay variability. The implication is such that if one were to develop, optimize and validate the assay on one pipetting platform (as is typically done), an assay transfer to automation may be challenging. This is relevant when considering setting appropriate specifications for controls or overall assay performance. It is not the intent of this paper to suggest testing every possible buffer in an assay, but instead to thoroughly evaluate the proposed assay buffer at the bench and ultimately on the intended liquid handler. This approach is equally important when one considers applying the same automation parameters to different assays with different constituents.

Second, when labware is not evaluated or reagent temperatures are not considered, the results of an assay can be affected, especially considering transfer from manual to automated or lab to lab. This was observed during the evaluation of plate type and effect of reagent temperature during pipetting. This is certainly not to say that all assays will be affected in the same manner as the model assay described in the report, however, it is incumbent upon the researcher to carefully study these types of variables.

Third, some parameters of the automated liquid handler affected the assay more than others depending on the buffer type employed. Specifically, three parameters (e.g., air gap volume, aspirate speed and dispense speed) were evaluated. Depending on assay buffer, some combinations had no effect, while others profoundly impacted potency, slope factor, span, variability, or combinations thereof. The overall implication of this finding is that seemingly minor changes to these parameters could lead one to make incorrect decisions when selecting compounds based on potency. Also, while one set of conditions may allow for optimal performance in a particular assay, those same parameters may not be translatable to another assay in which different buffers are used. Therefore, simply selecting a default automated liquid handling protocol is not advisable without studying the effects on each particular assay [22,28].

Fourth, by deconstructing an assay prior to optimization or automation validation and studying key parameters one can identify which parameters are likely to have an effect and which are not. It is important to reiterate that the findings for this model assay may not necessarily be the same as for other assays in screening campaigns, but instead it is the *approach* that can be translated into all assays destined for transfer.

Finally, as laboratories develop and use AI models to optimize automated liquid handlers, it is recommended to consider the entire assay process rather than just the individual liquid handler.

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