Introduction

The continual rise of drug prices and increased public scrutiny of pharmaceutical companies highlight the need for streamlining drug discovery operations. Advances in laboratory automation, including automated liquid handlers, have allowed high-throughput screening processes to rapidly evaluate a large number of compounds as potential drug candidates. Cost reductions have been achieved primarily through automation and miniaturization. One strategy to improve efficiency is through development of an effective quality control program. By implementing a robust liquid handling quality control program, drug discovery laboratories can build quality into their processes, decrease the overall cost by improving quality of data and shorten the drug discovery timeline as a result of reducing repeat testing or chasing false leads.

The liquid handling component of the overall assay variability can have a significant impact on the outcome of an assay. Many laboratories rely on precision alone to estimate the quality of the liquid handling. Furthermore, most labs optimize assays by examining individual assay assembly components. For example, liquid handlers (LHs) are typically verified offline, calibrated (and fixed, if necessary), then used.

Our hypothesis is that incubation, mixing, reader, plate movement, incubation and order of reagent addition all contribute to assay variability. We have previously shown that both liquid handling accuracy and precision is critical to assay performance. This study took the concept of optimization a bit further whereby the entire assay is considered. Process optimization includes measuring variability of the liquid handlers, mixing efficiency, incubation and order of reagent addition within the context of a process, rather than individually. By using this approach we were able to demonstrate additional reduction of assay variability using our model protein binding assay, streptavidin:biotin-Fluorescein.

Methods

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

Experimental Set-up

1) Add 25 µL of 30 nM biotin-Fl
2) Add 25 µL of buffer (inhibitor)
3) Add 25 µL of 9 nM streptavidin (SA)
4) Incubate for 15 min
5) Read fluorescence (Ex 485 nm, Em 515 nm)
6) Incubate and read every 15 min for 1 hour

Three liquid handlers (LHs) were used to dispense each of the assay reagents: Thermo Scientific Matrix WellMate, Beckman Biomek NX7, and Raninin Pipet-Lite manual 8-channel pipette.

This project was conducted in three phases

Phase 1: (a) Run assay on LHs to obtain assay results; (b) Test individual LHs with MVS to obtain volume results and optimize if necessary.

Phase 2: (a) Run assay on optimized LHs and obtain assay results; (b) Convert assay process to MVS readable configuration (Figure 2) to obtain volume accuracy and precision results as well as determine mixing efficiency and incubation time.

Phase 3: (a) Optimize liquid handling order and reagent addition with MVS to obtain volume accuracy and precision results; (b) Test optimized assay to obtain assay results.

Results

Figure 2: Basic scheme illustrating the dispense order test method, see results in Figure 3. This test was repeated to test the dispense order of the WellMate, Biomek and Multichannel.

Figure 3: Effect of reagent dispense order. When the WellMate is used to dispense reagent first, the imprecision is higher; a bulk dispenser should not be used to dispense the first reagent within the context of this assay process.

Table 1. Mixing efficiency and incubation time comparison. By using process optimization it was determined that a minimum of 30 minutes was needed for optimal assay signal. Alternatively, the assay obtained an equally optimal signal by employing a mixing step.

Conclusions

1. By studying the assay process, the reagent order was found to have an impact on assay variability. The variability (as measured by Z-factor) improved from a marginal 0.49 to 0.65, indicating an excellent assay.
2. Reagent mixing was also optimized. It was determined that the assay needed either a minimum of 30 minute incubation or thorough vortex mixing.

Overall Impact

Confidence in assay data can be increased by looking at how the individual LH components work together as a whole. By optimizing the assay process, including liquid handlers, mixing, incubation time, etc., the overall cost of the drug discovery process can be reduced by improving quality of data, removing unnecessary incubation or mixing steps and thus, shortening the drug discovery timeline.