Effect of Liquid Handling Quality Control on Biological Assay Performance

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Introduction

Molecules from a high throughput screening (HTS) campaign are often selected for further development after a single interrogation. HTS groups are also constantly challenged with improving efficiency by incorporating new technologies and complex biology. Hence, in order to obtain promising candidates, high quality assays are required. Assay variability is an important quality attribute and is a combination of liquid handling, biological and random variability. Liquid handling variability is often underappreciated, yet can have a huge impact on the outcome of an assay. Many laboratories rely on only precision to estimate the quality of the liquid handling. Optimization of liquid handling is so critical to improve the assay's performance that precision and accuracy must both be assessed. This study was designed to examine (1) liquid handling quality control (QC) tools and (2) the effect of liquid handling variability on a model biological assay.

The Artel MVS (Multichannel Verification System) was chosen as the tool for evaluating quality control because it is easily adaptable to automated liquid handling systems and because it effectively determines both precision and accuracy. This study evaluated six different plate types compatible with the Artel MVS. To minimize variability contribution from the liquid handler, a handheld single channel syringe pipette (eVol, SGE Analytical Science) was used to assess the performance of the plates. After testing a range of volumes (10 to 50 µL), it was determined that the Artel Verification plate performed best in terms of both accuracy (-0.1 to 0.74 % inaccuracy) and precision (0.15 to 0.3 %CV).

This experiment was then translated into an automated platform (Precision XS, Biotek) that better reflects a real scenario. The Precision XS performed well and the use of the Artel Verification plate yielded the best accuracy (0.02 % inaccuracy) and precision (as low as 0.2 %CV) for the MVS. Alternate off-the-shelf plate types used for liquid handling QC were also evaluated using the same liquid handler, detector and reagents. Drastically different accuracy and precision profiles were obtained which could lead to improper calibration of a liquid handler.

Finally, this study will demonstrate how an improperly calibrated liquid handler (LH) can affect the performance of a model biological assay. Data will be presented that demonstrates how only small changes in dispense volumes can affect inhibitor potency (IC50) values.

Ultimately, proper LH calibration is necessary for efficient assay transition points, such as from assay development to automation validation, primary screening to confirmation screening, lead generation to lead optimization, or simply replacing one liquid handler with another if failure occurs during a campaign.

Methods

Part A: Evaluation of LH Verification Tool

- Use Artel technology to measurement LH performance
- Examine 96-well, clear-bottom plates from five different sources
- Investigate different volumes:10, 20 and 50 µL
- Interested in variability due to plate, not liquid handler: eVol Digital Syringe (SGE Analytical Sciences), n = 16
- Compare to automated liquid handler: Precision XS (Biotek), n = 96
- Dispense Reagent A or B into empty plate wells using either eVol or Precision XS
- Add Diluent to total of 200 µL in each well
- Mix 1 minute
- Read absorbance at 530 and 720 nm
- Calculate dispense volume

Part B: Effect of LH Variability on Assay Performance:

- Develop model HT assay in 96-well format based on streptavidin-biotin
- Assay components
  - Phosphate buffered saline, pH 7.4, containing 0.1% BSA
  - Black, non-binding 96-well plates
  - Streptavidin (binding protein), 3 nM final
  - Biotin-fluorescein (labeled ligand), 10 nM final
  - Inhibitors
- Experimental conditions
  - Add 25 µL of 30 nM Biotin-FL
  - Add 25 µL of compound
  - Add 25 µL of 9 nM SA
  - Incubate for 60 min at room temperature
  - Read fluorescence, Ex = 485, Em 515
- Intentionally vary volume of each liquid handling step by <10%

Table 1: Liquid handler variability experimental set up used to generate Figures 5-8 and Table 2.

Plate ID	Biotin-Fl, µL	Cmpd, µL	SA, µL
1	25	25	25
2	23	23	23
3	27	27	27
4	23	27	27
5	23	25	27
6	27	23	25
7	23	23	27
8	25	27	23

Results

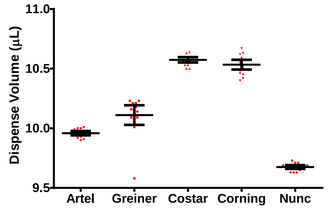

Figure 1. 10 µL dispense using eVol syringe pipette.

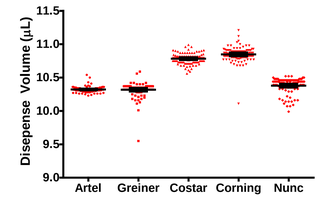

Figure 2. 10 µL dispense using Precision XS.

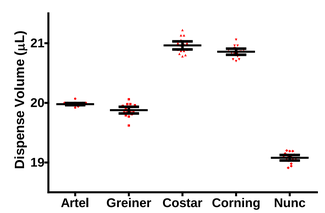

Figure 3. 20 µL dispense using eVol syringe pipette.

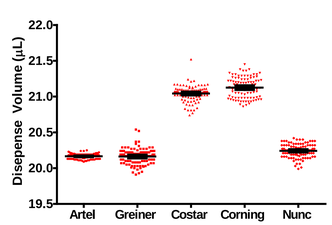

Figure 4. 20 µL dispense using Precision XS.

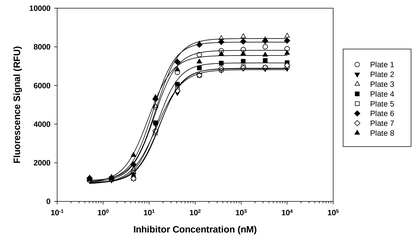

Figure 5. Effect of variability on biotin potency.

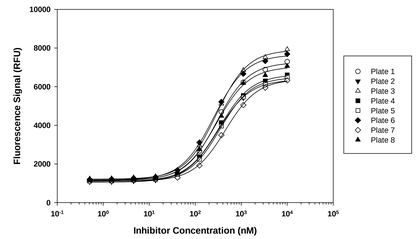

Figure 6. Effect of variability on desthiobiotin potency.

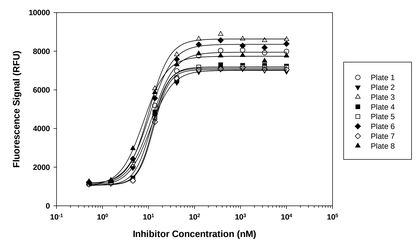

Figure 7. Effect of variability on biotin amino-hexanoic acid potency.

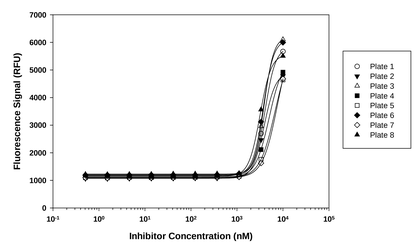

Figure 8. Effect of variability on iminobiotin potency.

Table 2: Liquid handler variability results summary.

	Plate Control Statistics				Inhibitor IC50, nM			
Plate ID	Min	Max	S/B	Z'	Biotin	Biotin-AH	DT-biotin	I-Biotin
1	1164	7694	6.61	0.956	12.13	10.38	325.5	4047
2	1064	6722	6.32	0.961	13.67	9.471	335.9	3922
3	1213	8354	6.89	0.955	12.78	8.747	312.6	3887
4	1106	7027	6.36	0.965	14.19	10.54	360.3	4997
5	1100	6920	6.29	0.942	15.96	10.90	381.4	6734
6	1187	8132	6.85	0.960	10.77	9.027	271.7	3740
7	1085	6814	6.28	0.960	15.76	11.33	504.7	7739
8	1142	7552	6.61	0.935	8.271	6.355	309.7	3166
Δ potency					8 nM	5 nM	233 nM	4573 nM

Conclusions

Part A: Evaluation of LH Verification Tool

1. Plate type does make a difference when using the MVS system for LH verifications
2. Artel Verification plates provide the best accuracy AND precision
3. An automated LH is sensitive to seemingly subtle differences in performance due to plate type

Part B: Effect of LH Variability on Assay Performance

1. LH variability does affect assay
   a. S/B is minimally affected
   b. Z-factor is not appreciably affected
   c. Compound potency is affected
2. Higher potency inhibitors seem less affected by LH variability
3. Lower potency inhibitors are more affected by LH variability

Overall Impact

- LH variability alone can have a negative impact on decisions, from primary screening to confirmation screening to SAR
- Minimizing erroneous data earlier in the lead generation process is less expensive than later
- By measuring LH accuracy and precision, assay transfer time can be minimized.

Work is currently underway to design a statistical approach to pair liquid handlers with each reagent in an automated screening platform. For example, reagents that are sensitive to minor variability would be dispensed on the best performing LH. Furthermore, by understanding the performance of each LH, it should be possible map out what LHs are interchangeable should a failure occur.