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Effect of Liquid Handling QC on Biological Assay Performance

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Artel Tutorial

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Outline

- Review accuracy vs. precision
- Review common liquid handler QC methods
- Artel MVS technology description
- Project scope

  Part I

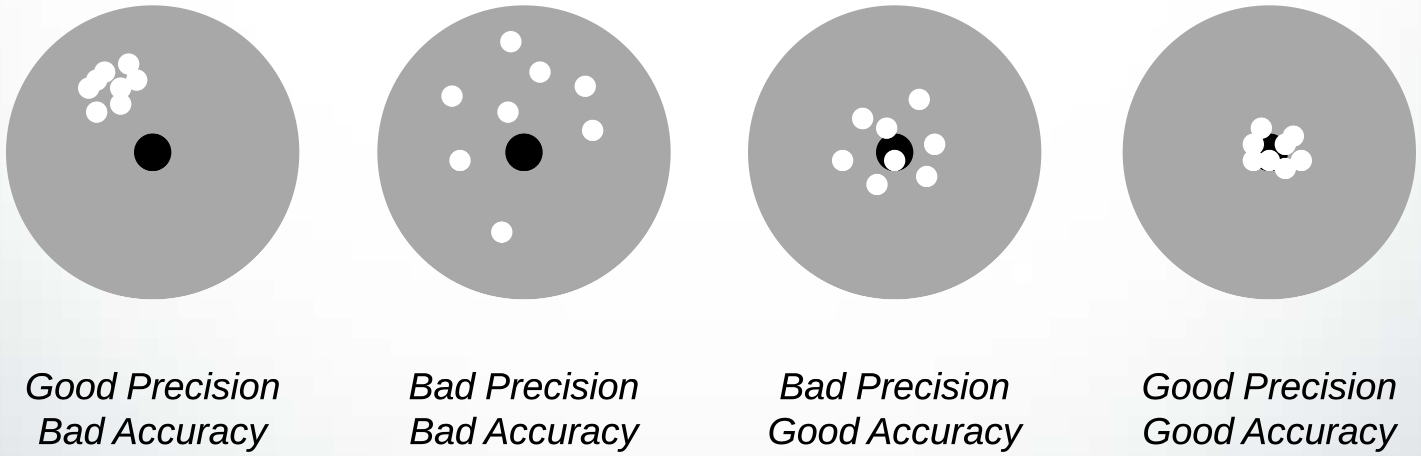
  - Use the best QC tool for the job
    - Investigate plate types
  - Understand baseline performance of the liquid handler (LH)

  Part II

  - Determine effect of LH variability on assay performance

Accuracy vs. Precision

- ***Accuracy****:* *The ability of a measurement to match the true value of the quantity being measured – Often measured as % relative inaccuracy*
- ***Precision****:* *The ability of a measurement to be consistently repeatable – Often measured as %CV or coefficient of variation*



Review of Liquid Handler QC Methods and Their Relative Strengths[1]

	<1μL	1-20 μL	>20 μL	Precision	Accuracy	Multi-channel	Alternative fluids
Gravimetric	-	-	+	-	+++	-	-
Absorbance (tartrazine)	-	+	++	+++	+	+++	+
Fluorescence (fluorescein)	++	+	++	+++	+	+++	-
Near Infrared[2] (977, 900 nm)	-	-	+	++	+	+++	-
Acoustic[3]	+++	+++	-	++	++	++	+
Dual-Dye ratiometric	++	+++	+++	+++	+++	+++	++

[1]Strengths are based on a variety of factors including complexity, detection limits, whether a calibration curve is needed, etc.
[2]Used by Molecular Devices and Biotek not necessarily for LH QC, but for pathlength correction to normalize well-to-well variability.
[3]Acoustic is mentioned due to prevalence of acoustic dispensing, not because it is a widely accepted method of QC.

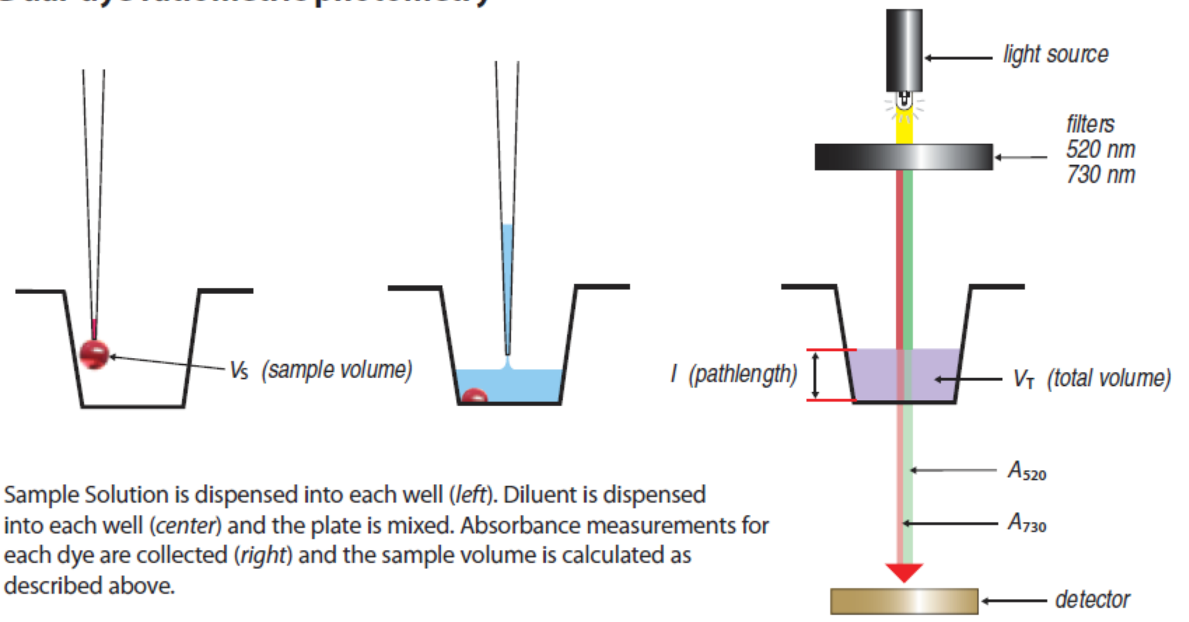
Artel MVS Technology

Three Calculations

1. Blue dye (730 nm) is used to determine pathlength: $l = A_{730}/a_b$
2. Well liquid volume (V_T) is calculated using l and well dimensions θ and D
3. Red dye (520 nm) used to determine Sample volume (V_S) is calculated using:

$$V_S = V_T \left(\frac{a_b}{a_r} \right) \left(\frac{A_{520}}{A_{730}} \right)$$

Dual-dye ratiometric photometry



Sample Solution is dispensed into each well (*left*). Diluent is dispensed into each well (*center*) and the plate is mixed. Absorbance measurements for each dye are collected (*right*) and the sample volume is calculated as described above.

Common Sources of Variability for Calibration Measurements

- Pipettor
- Reagents
- Tips
- Temperature
- Operator

These contribute directly to the liquid dispensation variability

- Plate type
- Mixing
- Detector

These affect the measurement process

Part I: Objectives

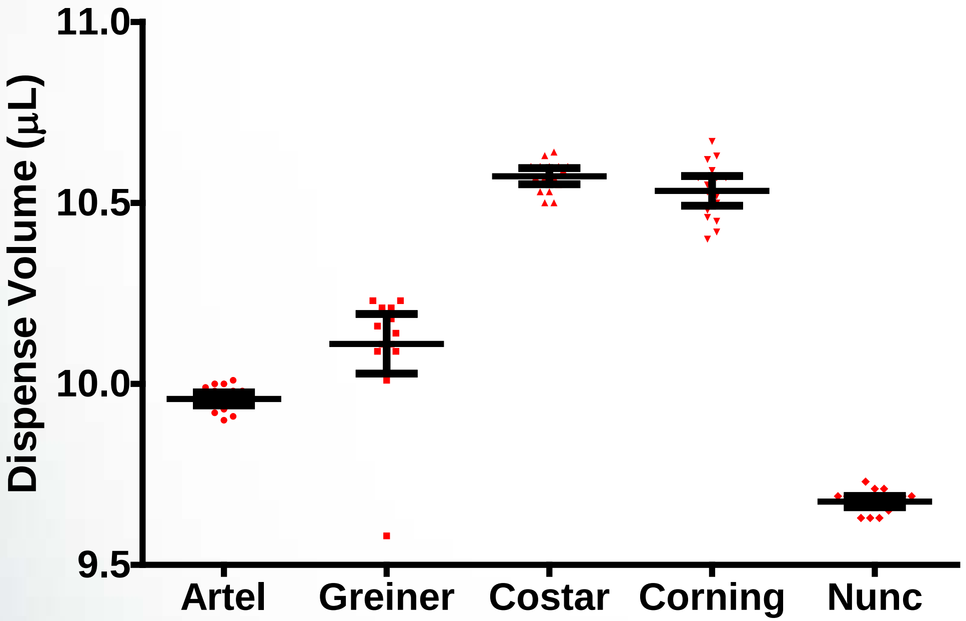
Establish baseline performance of automated LH

- Identify best QC method
- Investigate plate types
- Minimize sources of variability
  - Artel MVS reader is well characterized
  - Artel MVS reagents are well characterized and NIST traceable
  - eVol digital syringe is a direct displacement dispenser that has been gravimetrically calibrated
  - Temperature held constant at 22.2 ± 0.1 °C
- Test LH to determine performance

Part I: Experimental Summary

- 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

eVol Syringe: 10 µL Dispense



- Same pipette
- Same tip
- Same reagents
- Same detector
- Different plate types

Plate ID	Artel	Greiner	Costar	Corning	Nunc
Target Volume (µL)	10	10	10	10	10
Number of data points	16	16	16	16	16
Mean volume (µL)	9.96	10.11	10.57	10.53	9.68
Relative Inaccuracy	-0.41%	1.11%	5.74%	5.33%	-3.25%
Standard Deviation (µL)	0.034	0.155	0.042	0.078	0.030
Coefficient of Variation	0.35%	1.53%	0.40%	0.74%	0.31%

eVol Syringe: 20 µL Dispense

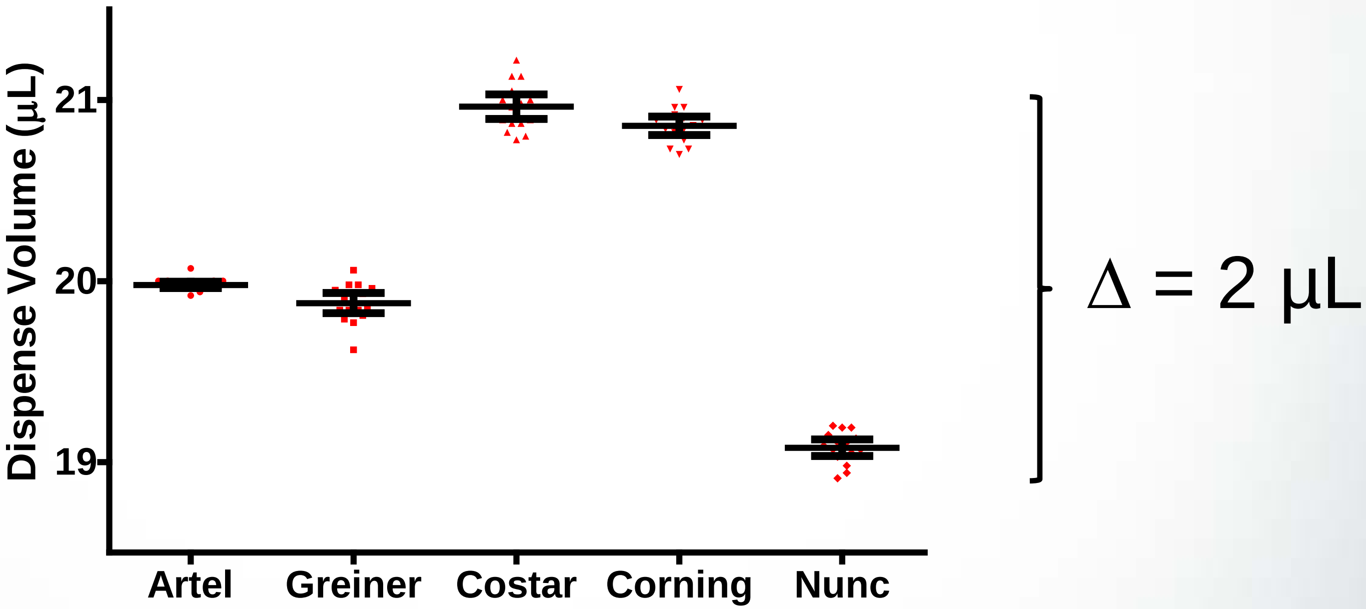


Plate ID	Artel	Greiner	Costar	Corning	Nunc
Target Volume (µL)	20	20	20	20	20
Number of data points	16	16	16	16	16
Mean volume (µL)	19.98	19.88	20.96	20.86	19.08
Relative Inaccuracy	-0.11%	-0.61%	4.82%	4.29%	-4.60%
Standard Deviation (µL)	0.033	0.105	0.128	0.095	0.086
Coefficient of Variation	0.17%	0.53%	0.61%	0.46%	0.45%

eVol Syringe: 50 µL Dispense

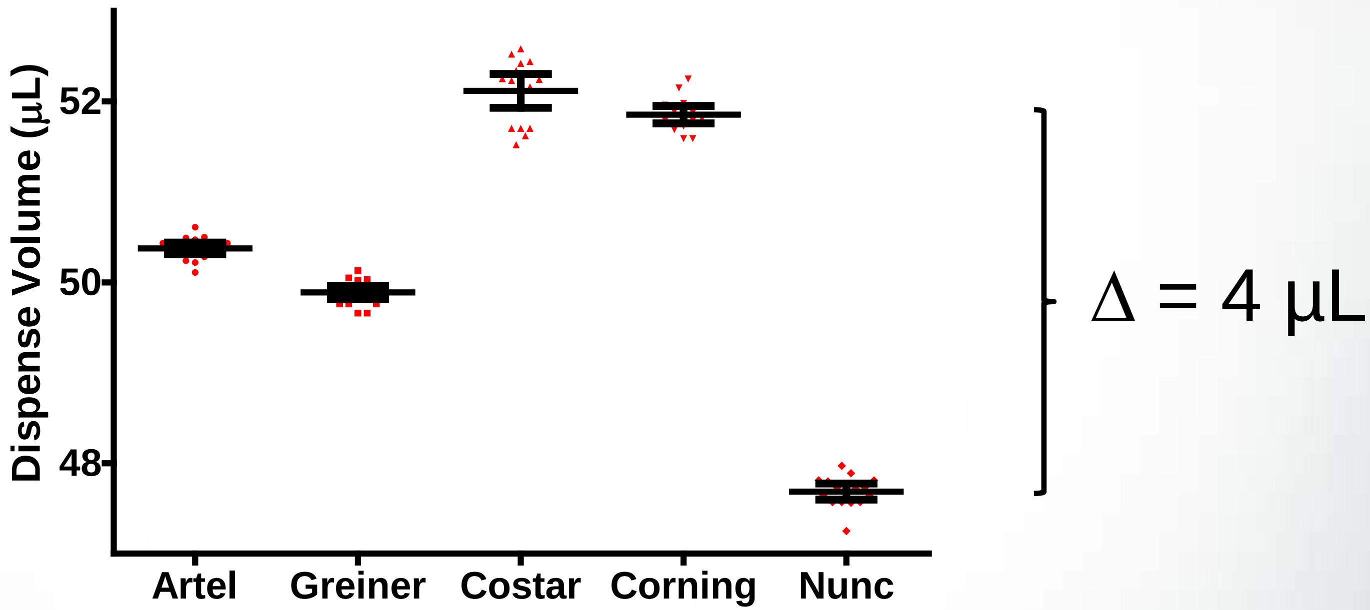


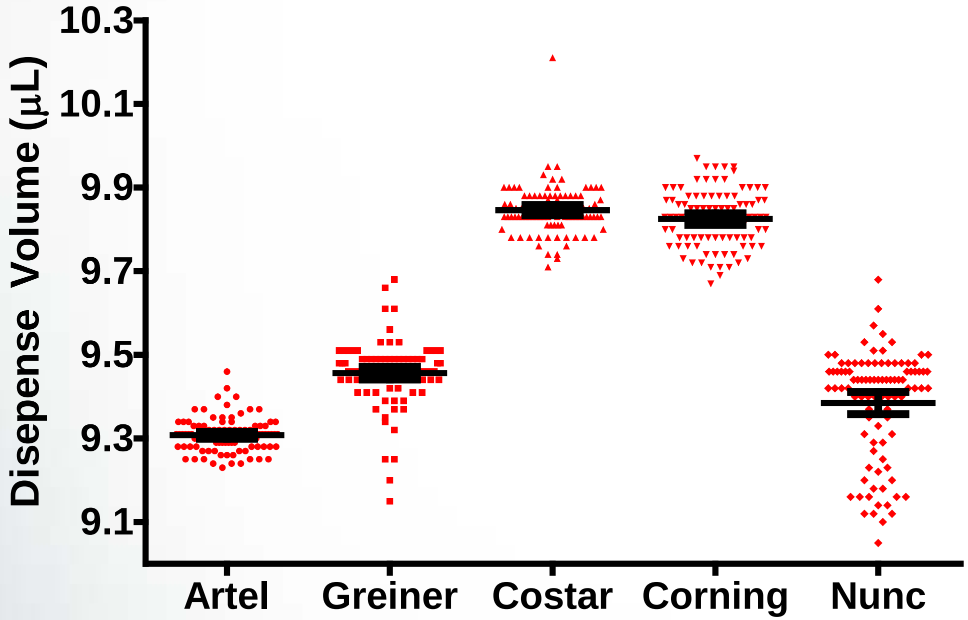
Plate ID	Artel	Greiner	Costar	Corning	Nunc
Target Volume (µL)	50	50	50	50	50
Number of data points	16	16	16	16	16
Mean volume (µL)	50.37	49.89	52.12	51.85	47.69
Relative Inaccuracy	0.75%	-0.22%	4.23%	3.70%	-4.63%
Standard Deviation (µL)	0.123	0.139	0.349	0.181	0.168
Coefficient of Variation	0.24%	0.28%	0.67%	0.35%	0.35%

Plate Type Comparison

- There is a clear difference in performance of plate types
- For best results (accuracy AND precision) Artel Verification plates need to be used
- OK, but what about a more realistic liquid handler?

→ Repeat experiment using Precision XS automated LH

Precision XS: 10 μL (as is)



- Different pipette tip for each well
- Same reagents
- Same detector
- Different plate types

	Artel	Greiner	Corning	Costar	Nunc
Target Volume (μL)	10	10	10	10	10
Number of data points	96	96	96	96	96
Mean volume (μL)	9.31	9.46	9.85	9.82	9.38
Relative Inaccuracy	-6.90%	-5.40%	-1.50%	-1.80%	-6.20%
Standard Deviation (μL)	0.04	0.08	0.06	0.07	0.13
Coefficient of Variation	0.43%	0.85%	0.61%	0.71%	1.39%

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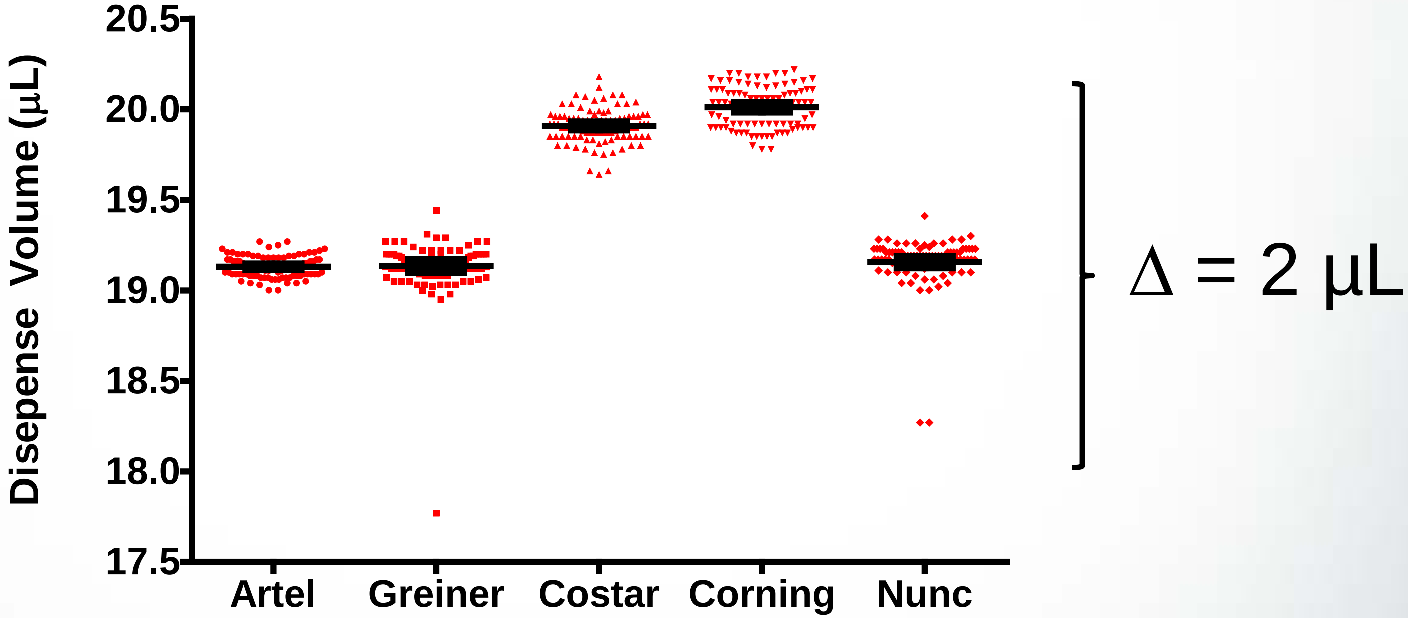
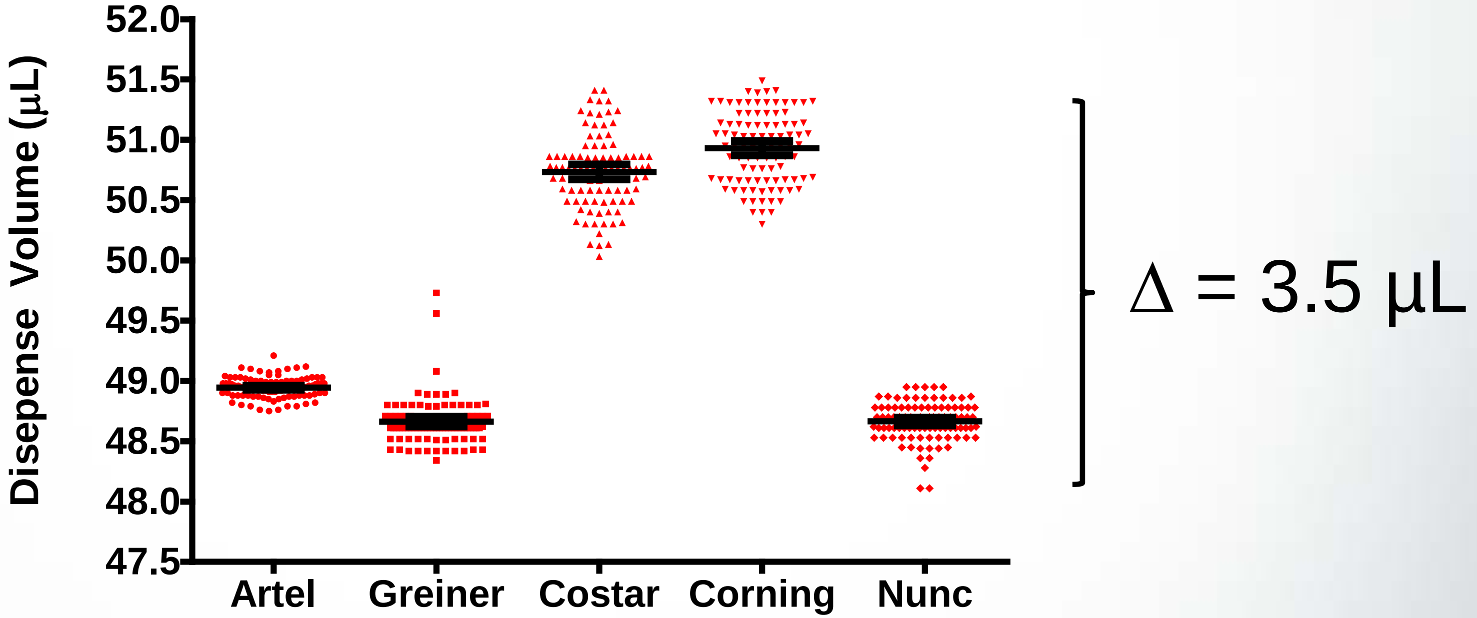
Precision XS: 20 µL (as is)



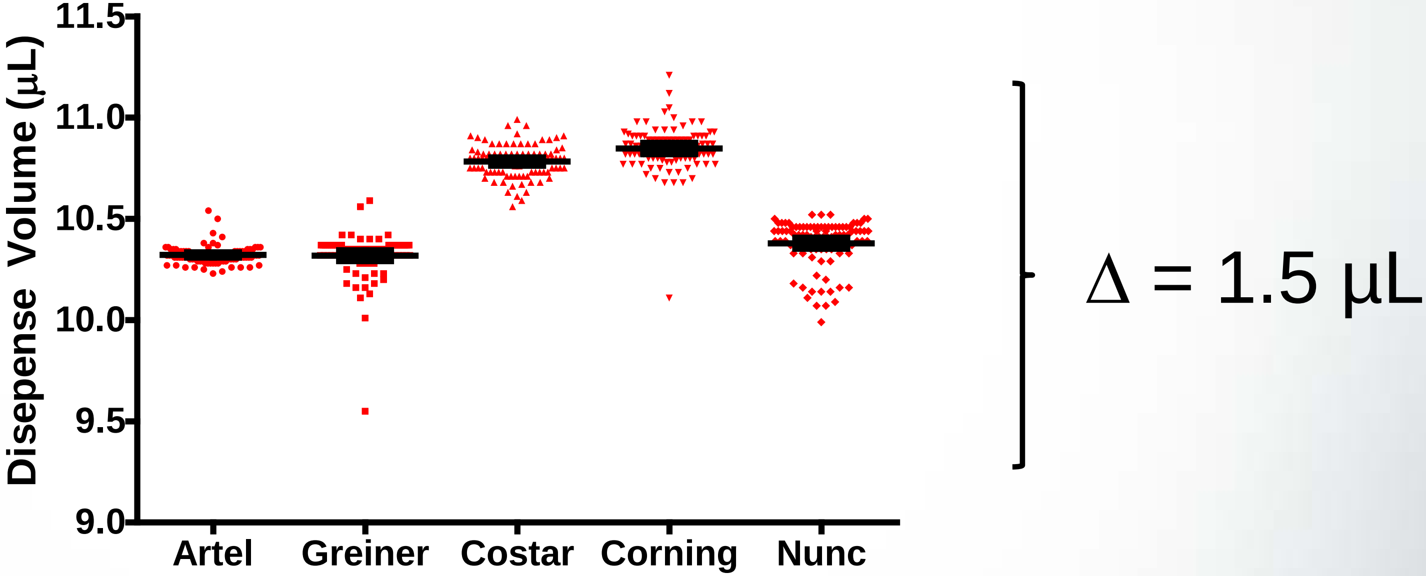
	Artel	Greiner	Corning	Costar	Nunc
Target Volume (µL)	20	20	20	20	20
Number of data points	96	96	96	96	96
Mean volume (µL)	19.13	19.13	19.91	20.01	19.16
Relative Inaccuracy	-4.35%	-4.35%	-0.45%	0.06%	-4.20%
Standard Deviation (µL)	0.06	0.16	0.10	0.12	0.15
Coefficient of Variation	0.31%	0.84%	0.50%	0.58%	0.78%

Precision XS: 50 µL (as is)



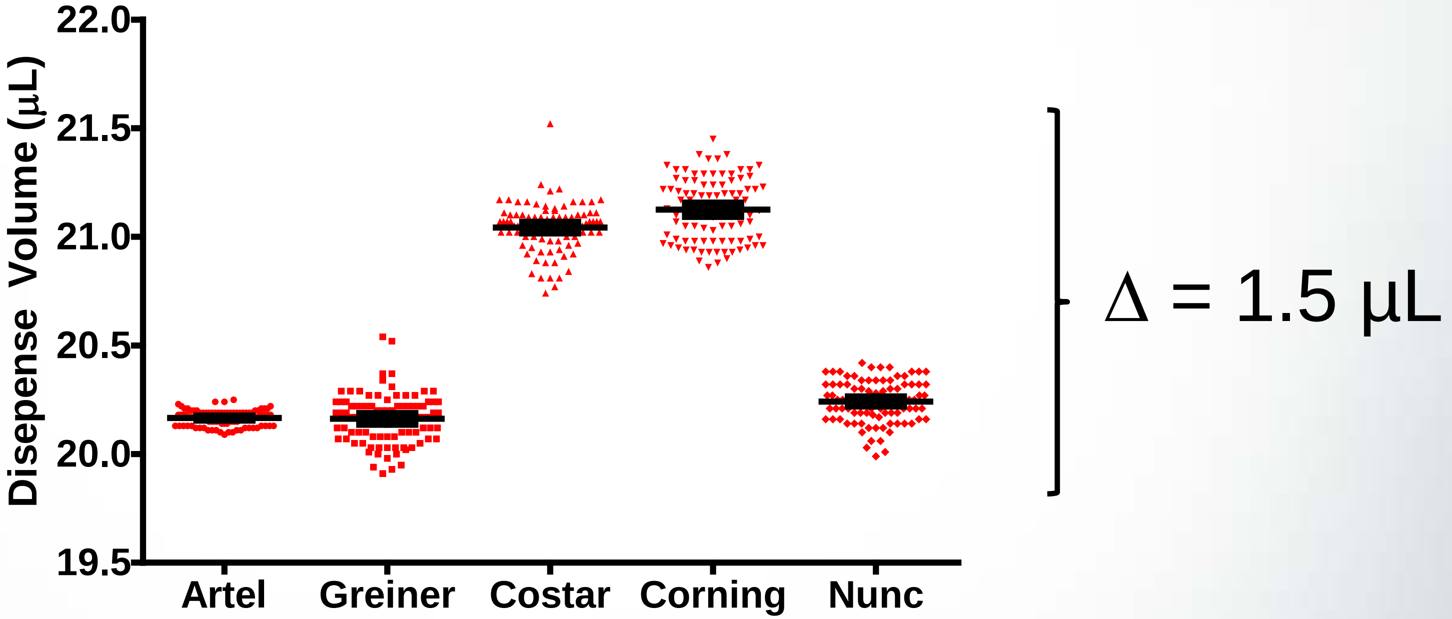
	Artel	Greiner	Corning	Costar	Nunc
Target Volume (µL)	50	50	50	50	50
Number of data points	96	96	96	96	96
Mean volume (µL)	48.95	48.66	50.73	50.93	48.66
Relative Inaccuracy	-2.10%	-2.68%	1.46%	1.86%	-2.68%
Standard Deviation (µL)	0.09	0.2	0.3	0.3	0.17
Coefficient of Variation	0.18%	0.41%	0.59%	0.59%	0.35%

Precision XS: 10 μL (adjusted)



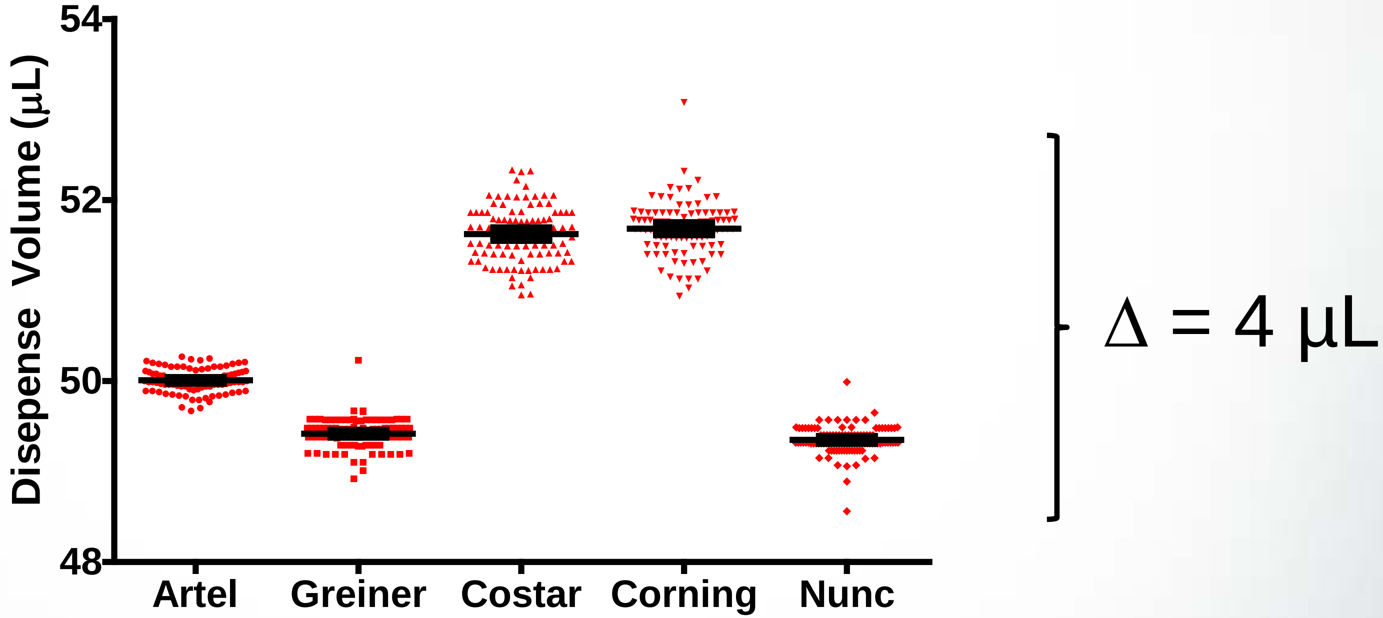
	Artel	Greiner	Corning	Costar	Nunc
Target Volume (μL)	10	10	10	10	10
Number of data points	96	96	96	96	96
Mean volume (μL)	10.32	10.32	10.78	10.85	10.38
Relative Inaccuracy	3.20%	3.20%	7.80%	8.50%	3.80%
Standard Deviation (μL)	0.05	0.11	0.08	0.12	0.12
Coefficient of Variation	0.48%	1.07%	0.74%	1.11%	1.16%

Precision XS: 20 µL (adjusted)



	Artel	Greiner	Corning	Costar	Nunc
Target Volume (µL)	20	20	20	20	20
Number of data points	96	96	96	96	96
Mean volume (µL)	20.17	20.16	21.04	21.12	20.24
Relative Inaccuracy	0.85%	0.80%	5.20%	5.60%	1.20%
Standard Deviation (µL)	0.04	0.11	0.11	0.14	0.09
Coefficient of Variation	0.20%	0.55%	0.52%	0.66%	0.44%

Precision XS: 50 μL (adjusted)



	Artel	Greiner	Corning	Costar	Nunc
Target Volume (µL)	50	50	50	50	50
Number of data points	96	96	95	96	96
Mean volume (µL)	50.01	49.42	51.62	51.68	49.35
Relative Inaccuracy	0.02%	-1.16%	3.2%	3.36%	-1.30%
Standard Deviation (µL)	0.13	0.17	0.32	0.3	0.17
Coefficient of Variation	0.26%	0.34%	0.6%	0.58%	0.34%

Part I: Conclusions

- Plate type does make a difference when using the MVS system for LH verifications; data is consistent between two independent LHs
- Artel Verification plates provide the best accuracy AND precision
- An automated LH can detect seemingly subtle differences in performance due to plate type
- Precision XS LH is capable of dispensing with better than 0.5% CV

Part II: Objectives

Investigate effect of LH variability on assay performance

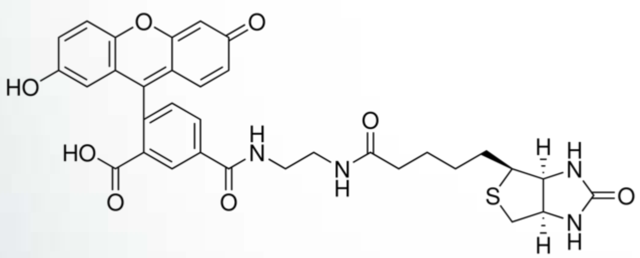
- Develop a model high throughput assay
  - Validate and characterize
- Characterize performance of various LHs using model assay
- Intentionally vary volume of each assay component to determine effect on various inhibitor IC50s

Part II: Experimental Summary

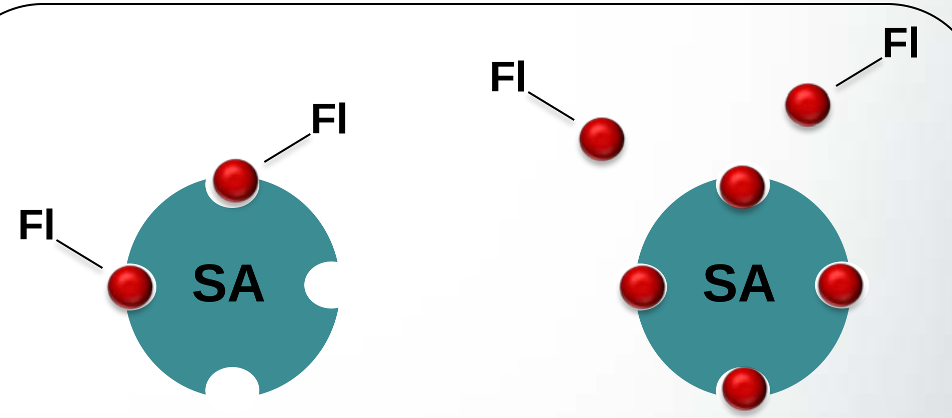
- Develop model HT assay in 96-well format
- Total assay volume = 75 µL; 25 µL for each of three LH additions
- Intentionally vary volume of each liquid handling step by <10%
- Measure effect by comparing dose-response (IC50s)
  - 10-point dose-response curves, n= 3

Streptavidin : Biotin-Fl Assay Principle

Streptavidin (SA) is a tetrameric biotin-binding protein that is isolated from *Streptomyces avidinii* and has a mass of 60.0 kDa. SA has a very high affinity for biotin ($K_d = 10^{-14}$ to $^{-15}$M).



Biotin-Fluorescein (B-Fl)

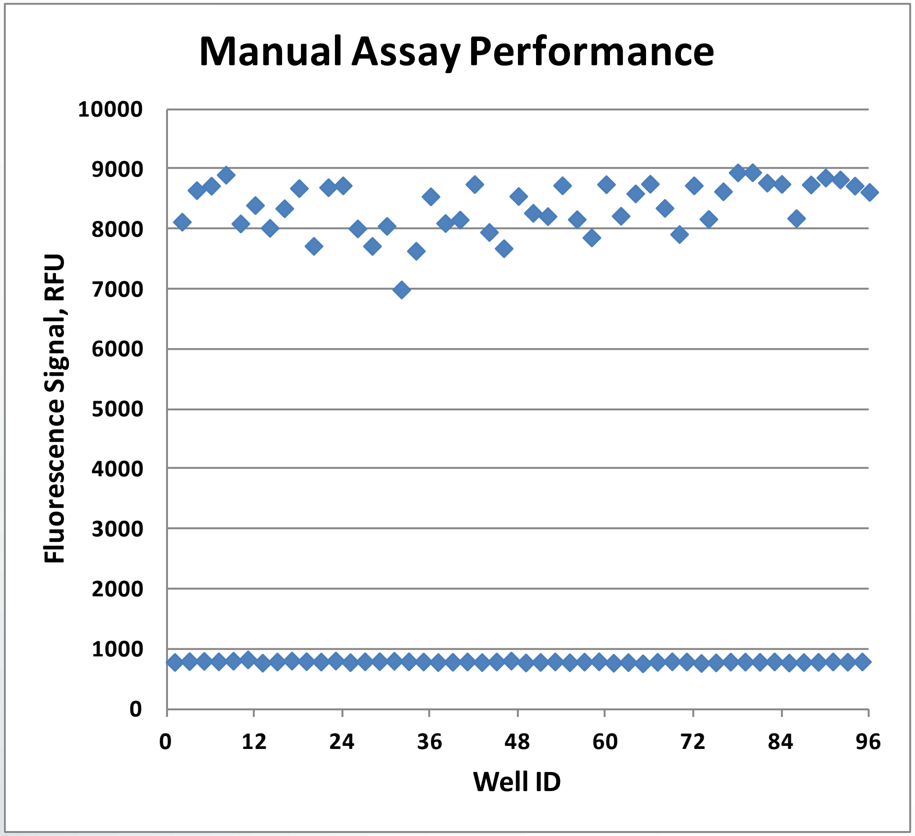


1. Waner, MJ; Mascotti DP. *Journal of Biochemical and Biophysical Methods* 70(6), 2008, 873-877. A simple spectrophotometric streptavidin–biotin binding assay utilizing biotin-4-fluorescein.
2. Ebner, A; Marek, M; Kaiser, K; Kada, G; Hahn, CD; Lackner, B; Gruber, HJ. *Methods in Molecular Biology*, 418, 2008, 73-88. Application of biotin-4-fuorescein in homogeneous fluorescence assays for avidin, streptavidin, and biotin or biotin derivatives.

Model HTS Assay

- 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 and Em 515

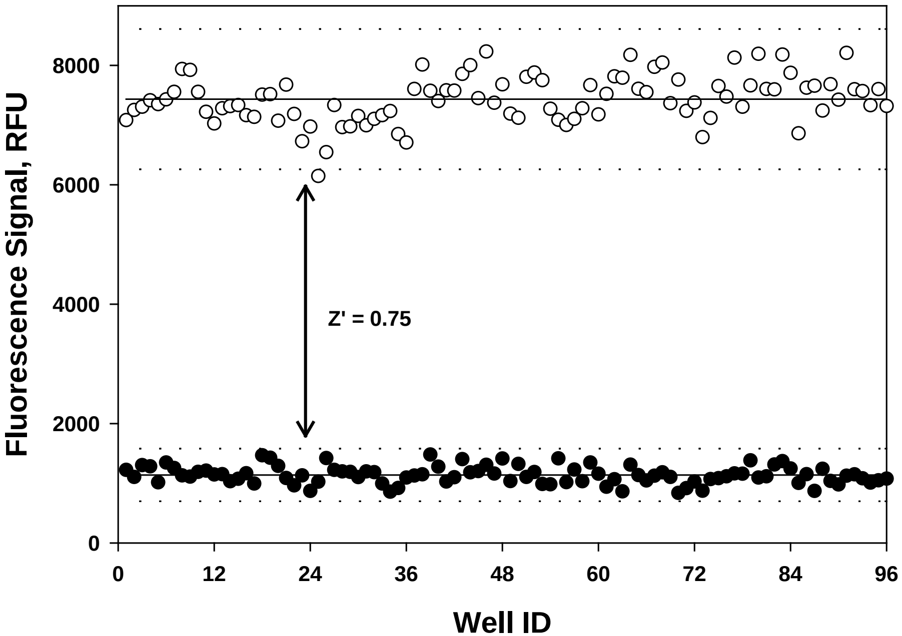
Assay Development: Manual Pipetting



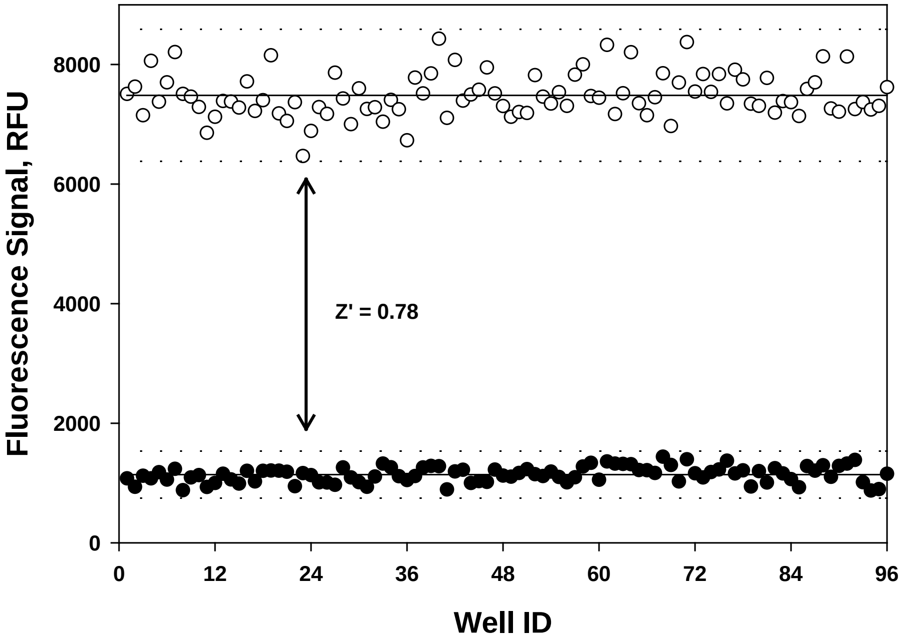
Min: 790 ± 12 (1.5%)
Max: 8376 ± 428 (5.1%)
Z-factor: 0.826
n = 48 for Max and Min

Assay Development: Peristaltic Dispenser

Min-Max 1

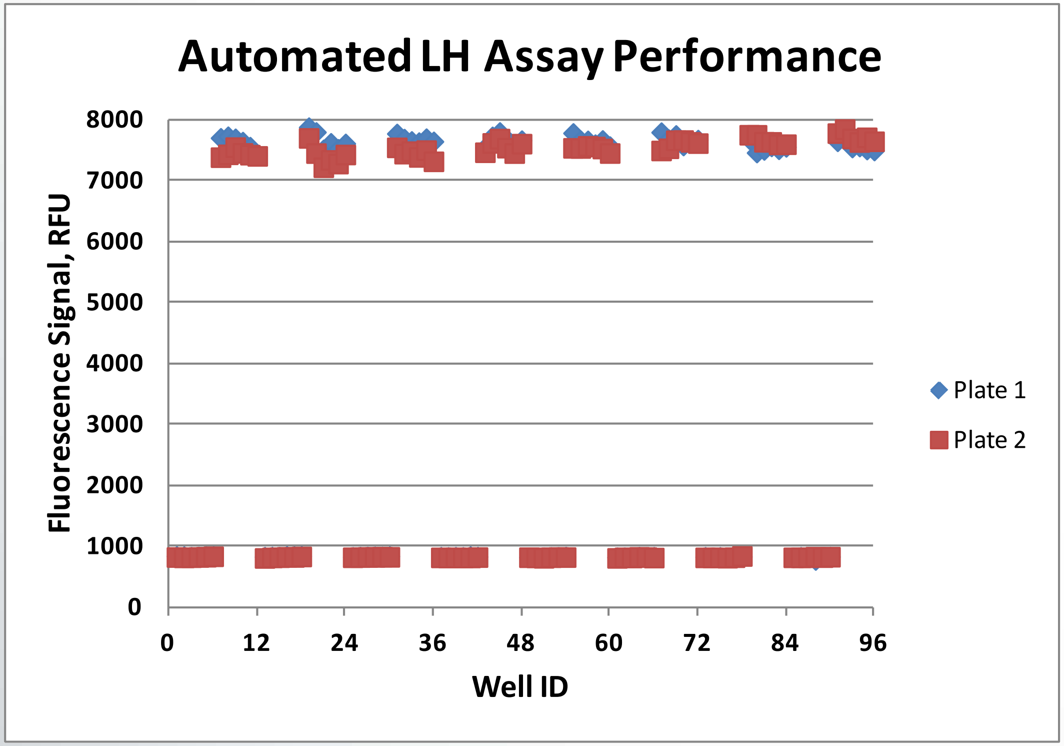


Min-Max 2



n = 96 for Max and Min

Assay Development: Precision XS



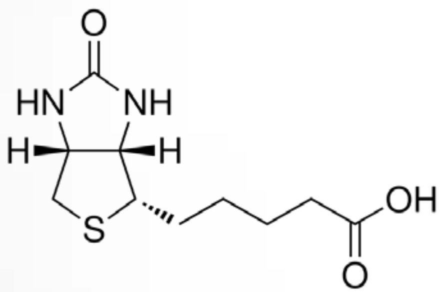
Min: 826 ± 10 (1.2%)
Max: 7633 ± 100 (1.3%)
Z-factor: 0.951
n = 48 for Max and Min

Min: 819 ± 6 (0.8%)
Max: 7543 ± 135 (1.8%)
Z-factor: 0.937
n = 48 for Max and Min

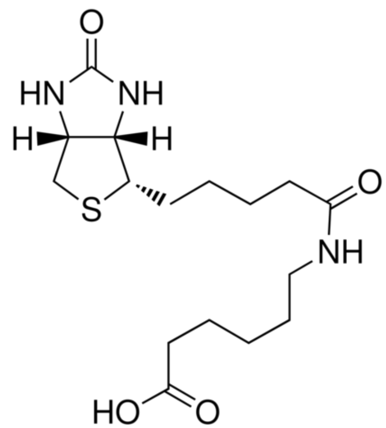
LH Variability Experimental Set-up

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

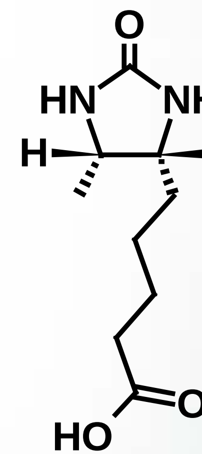
Inhibitors Studied



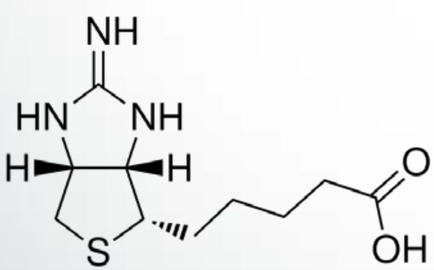
Biotin



Biotin Aminohexanoic Acid (Biotin-AH)

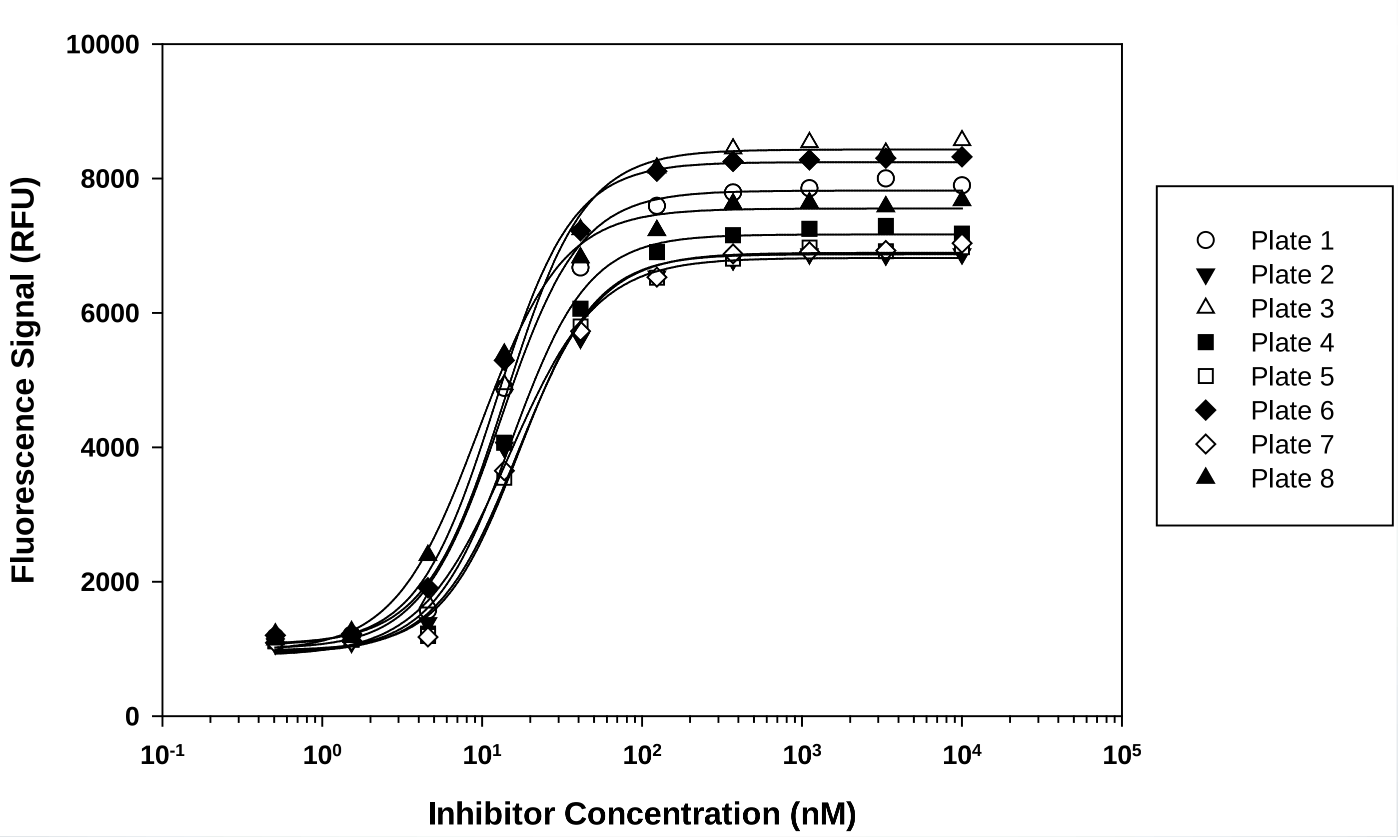


Desthiobiotin (DT-Biotin)

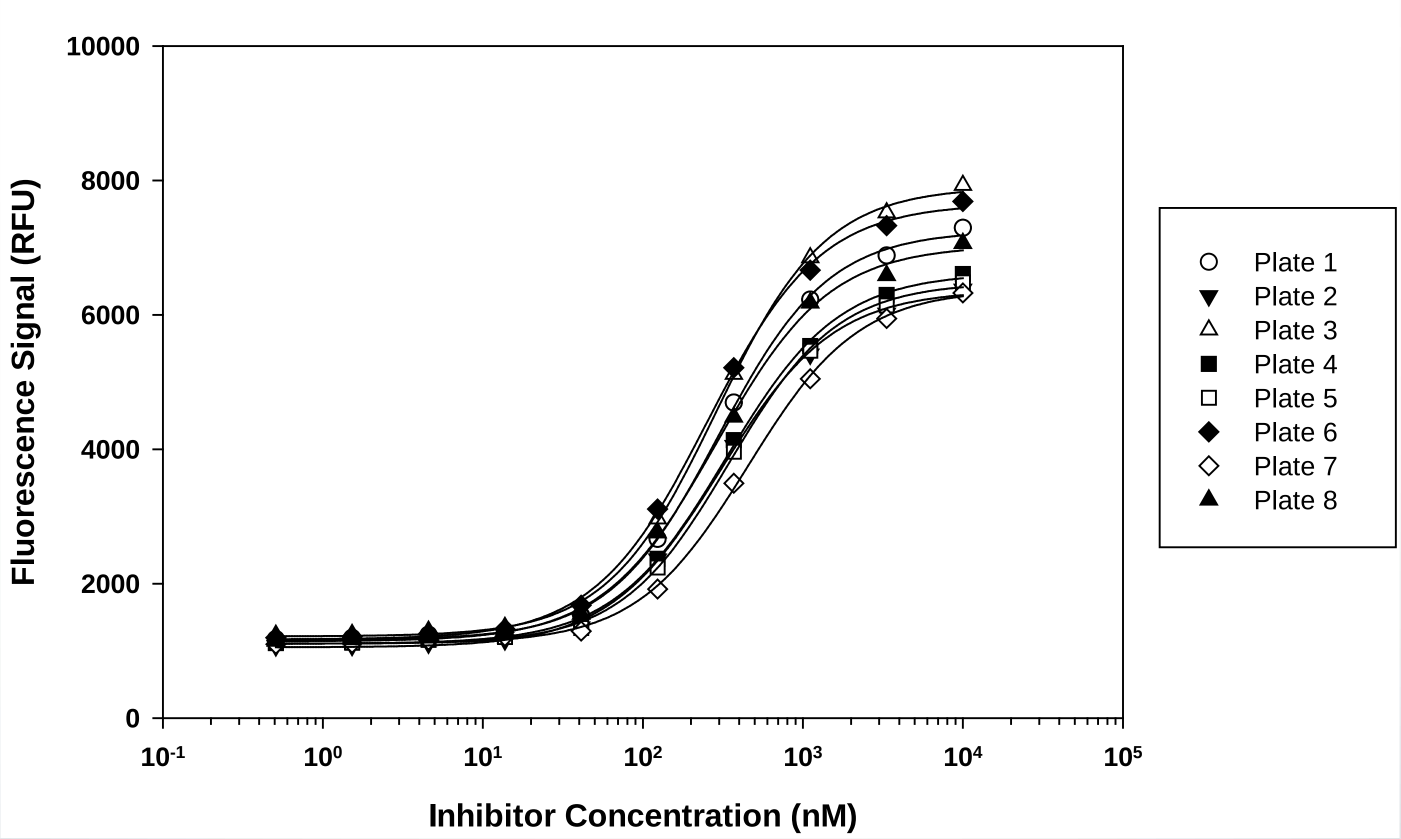


Iminobiotin (I-Biotin)

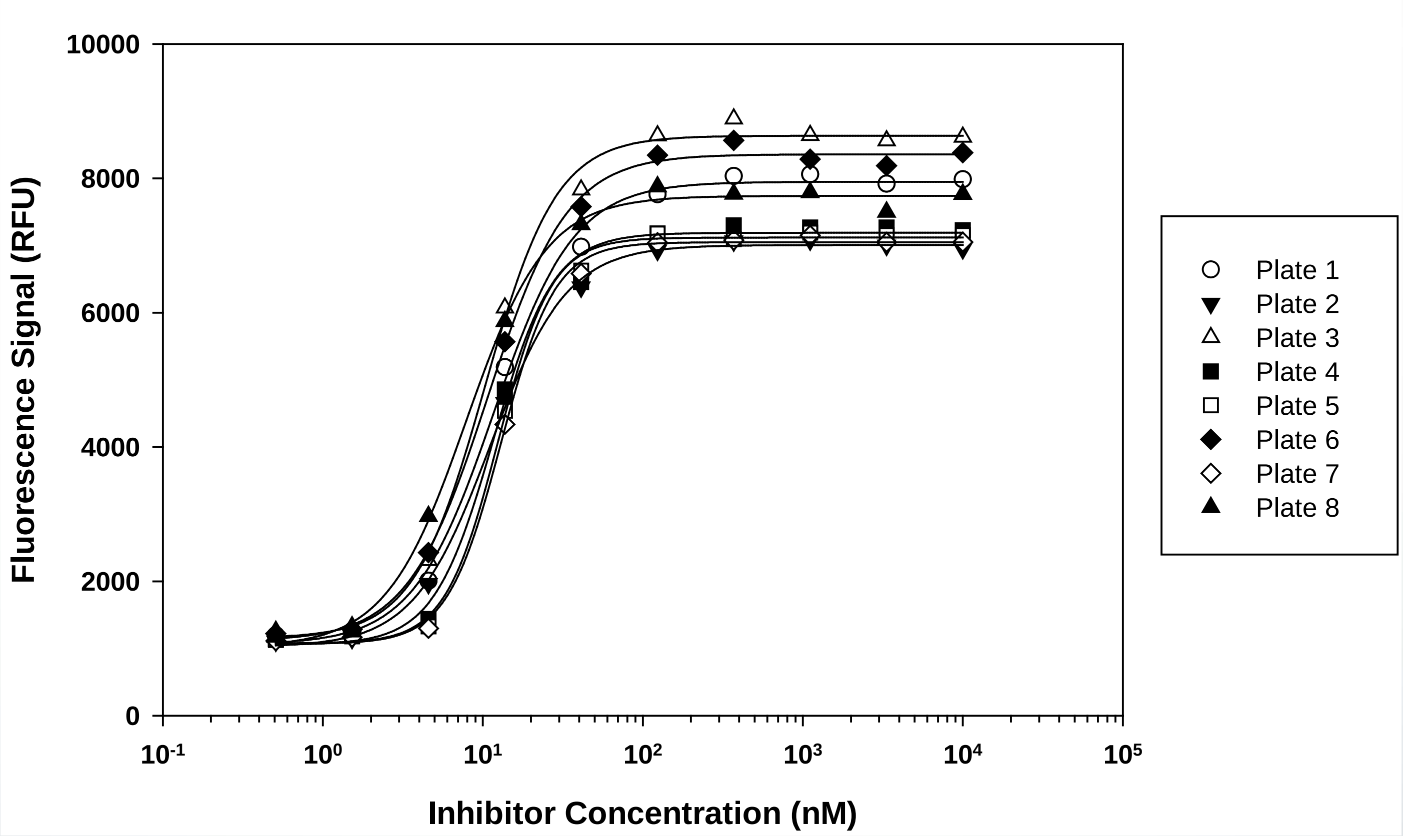
Effect of Variability on Biotin Potency



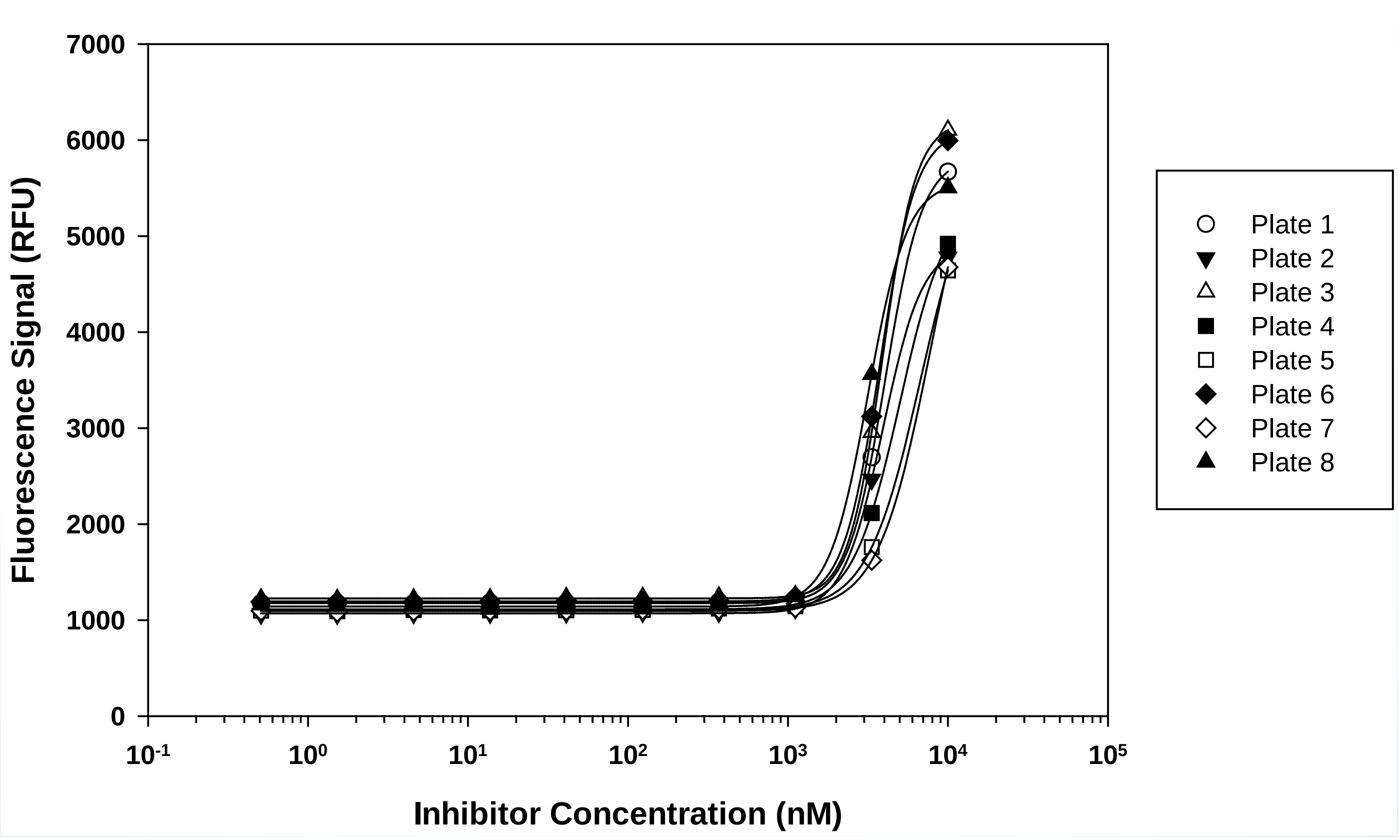
Effect of Variability on Desthiobiotin Potency



Effect of Variability on Biotin Aminohexanoic Acid Potency



Effect of Variability on Iminobiotin Potency



Part II: Summary of Results

	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

Part II: Conclusions

- LH variability does affect assay
  - S/B is minimally affected
  - Z-factor is not appreciably affected
  - Compound potency is affected
- Higher potency inhibitors seem less affected by LH variability
  - Resolution between similar potency compounds is decreased
- Lower potency inhibitors are more affected by LH variability
  - Can lead to missing important chemical scaffolds

Project Summary

- Using the correct tool is a must for LH performance verification
- 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
- LH variability can affect negatively impact assay transfer (e.g., development → automation, HTS → confirmation, confirmation → LG/LO) and replacement LHs
- Work is underway to experimentally match appropriate LH to critical reagent(s)

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