ARTEL

SLAS2019 Tutorial: Coupling Assay Design And Process Optimization Toward Minimizing Variability

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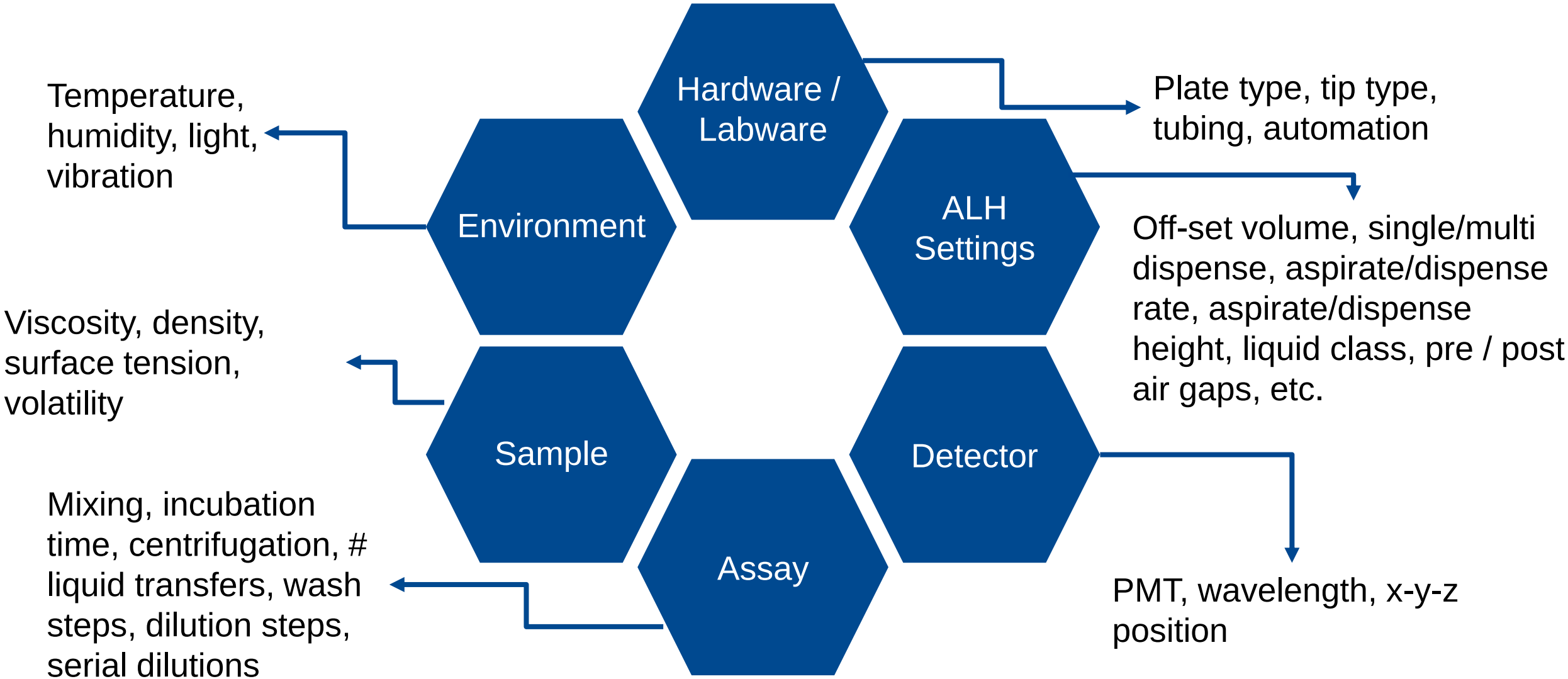
Acknowledgment: Becky Kitchener, PhD

Tutorial Agenda

- Assay variability
- Liquid handler optimization: current practices
- Process optimization – What? Why? How?
- Case study: model assay & findings
- Conclusions

OVERVIEW: ASSAY VARIABILITY

Parameters that Effect Assay Variability



Assay Validation in a High-Throughput World

So, What Does A "Good" Assay Look Like?

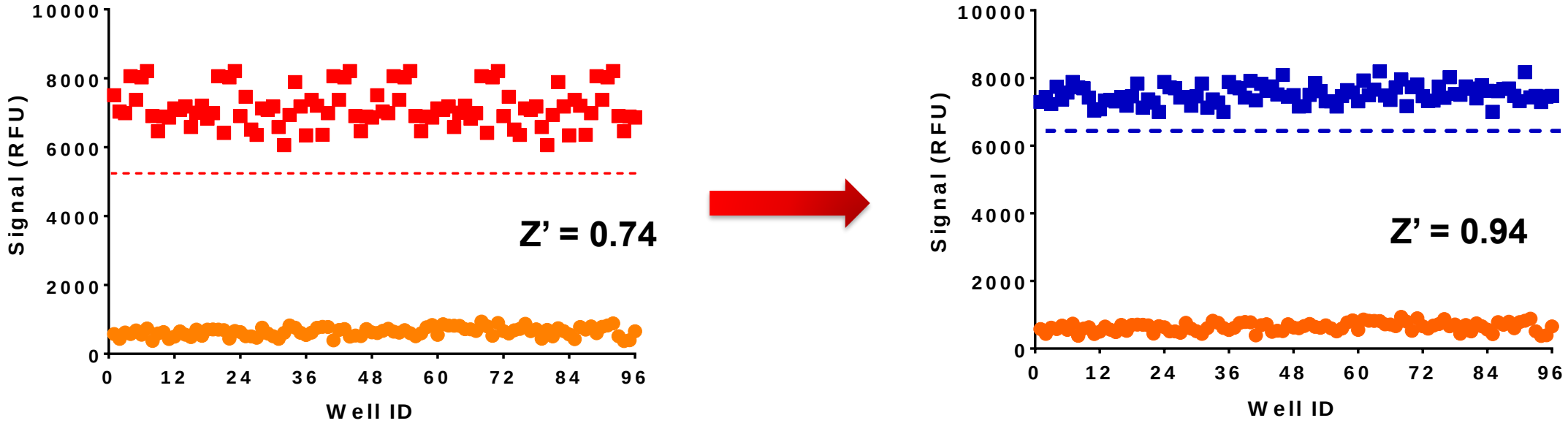
Let's define a couple of parameters:

- First, let's consider the assay variability
- Second, let's consider the assay window

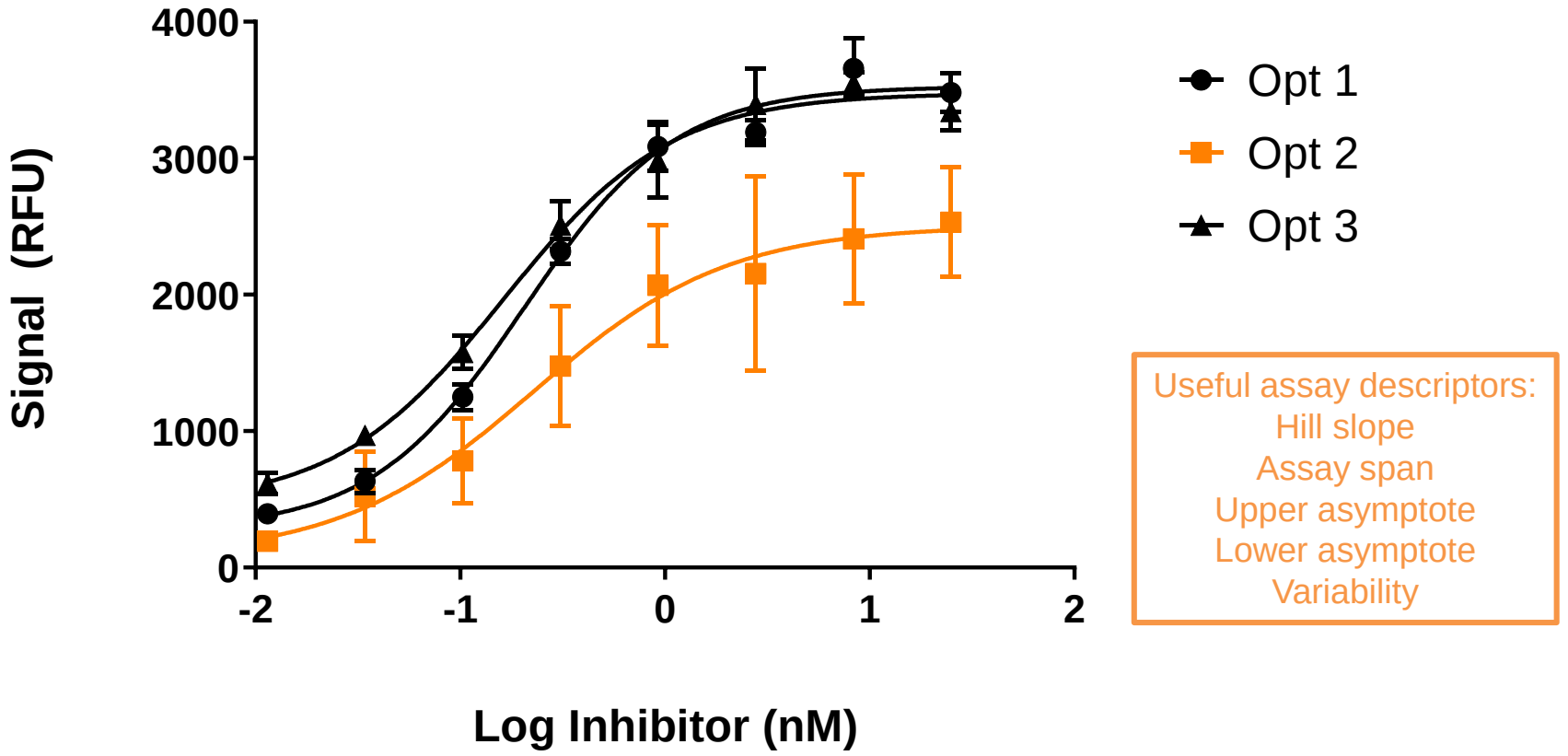
$$Z = 1 - \left(\frac{3\sigma_{\max} + 3\sigma_{\min}}{\left| \mu_{\max} - \mu_{\min} \right|} \right)$$

Z-Factor Value	Assay Quality
1	Ideal assay
1 > Z ≥ 0.5	Excellent assay
0.5 > Z > 0	Marginal assay
0	Yes/no assay
<0	Assay not useful

The Result



The Result



Useful assay descriptors:
Hill slope
Assay span
Upper asymptote
Lower asymptote
Variability

OVERVIEW:
LIQUID HANDLER OPTIMIZATION

Liquid Dispense Technologies

Dispense Technology	Attributes
Air displacement	• Problematic for volatile liquids • Possible cross contamination • Wide range of volumes
Positive displacement	• Useful for volatile solvents • Cross contamination possible • Wide range of volumes
Droplet (acoustic)	• Non-contact • Useful for small volumes (pL – nL)
Droplet (solenoid/inkjet)	• Useful for small volumes (nL) • Sensitive to fluid types
Capillary (pintool)	• Useful for small volumes • Direct contact with sample (contamination) • Sensitive to fluid type
Peristaltic	• Useful for bulk dispense; • More frequent calibrations needed

Parameters that Effect Volume Transfer

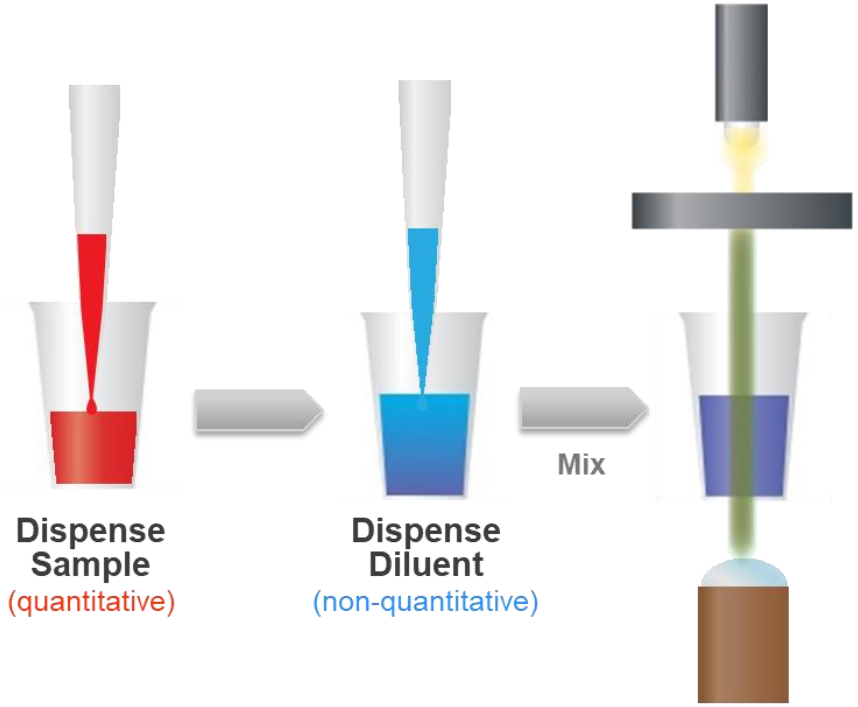
- **Hardware/labware** – plate format, tip types, tubing type
- **ALH settings** – target (or off-set) volume, single/multi dispense, aspirate/dispense rate, aspirate/dispense height, liquid class, pre and post air gaps, accuracy/precision of volume transfer, transfer speed/time delays, on-board mixing
- **Assay** – reagent mixing, incubation, centrifugation, number of liquid transfers, wash steps, dilution steps, serial dilutions
- **Sample** – viscosity, density, surface tension, temperature, volatility
- **Environment** – temperature, humidity, light, vibration

Typical Liquid Handler Optimization

- Usually performed as a stand-alone activity
  - Precision is always checked, but accuracy is not as easy
- Several methods for volume verification
  - In-house (e.g., fluorescence, gravimetric, absorbance, etc.)
  - Commercial (e.g., dual-dye spectrophotometry)
- Volume verification is typically performed with ideal solutions
- Liquid handler is certified, calibrated, or repaired (if necessary)
- Then….someone programs ALH for assay use
  - Default method
  - Specific to basic assay requirements

Artel MVS: A Useful Tool

- Employs a dual-dye, dual-wavelength, ratiometric absorbance-based measurement method for calculating the dispense volume.
- How it works: dyes of known concentration are dispensed into well-characterized microtiter plate. The plate is mixed on a plate shaker to ensure solution homogeneity. Absorbance readings are taken at 520 nm and 730 nm.



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

ADOPTING A PROCESS OPTIMIZATION APPROACH

Sources of Assay Variability

What can we control or optimize?

Biology

- Diffusion
- Binding equilibrium
- Steric hindrance
- Cell population diversity
- Protein activity

Environment

- ✓ Temperature
- ✓ Light
- ✓ Humidity
- People

Instrumentation

- ✓ Liquid handlers
- ✓ Detectors
- ✓ Mixers
- ✓ Incubators
- ✓ Centrifuges

Consumables

- ✓ Reagents
- ✓ Tips
- ✓ Plate type
- ✓ Plate seals

Artel MVS is More Than a Calibration Tool

CASE STUDY

Assessing Effects of Liquid Handling on the Assay

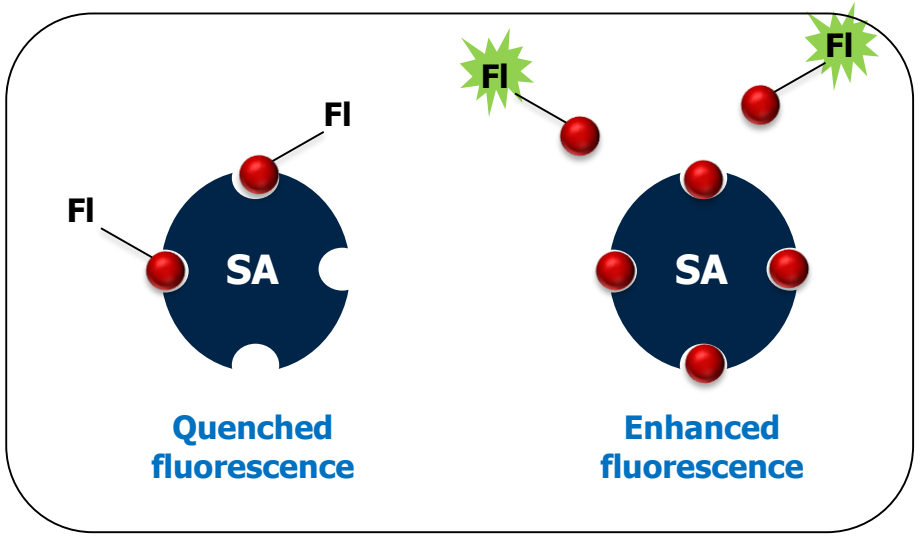
- **Phase I**: Using a well-characterized model assay, develop and optimize the assay platform at bench scale.
- **Phase II**: Perform method transfer to ALH: examine effects of automated liquid handling parameters on the same assay.
- **Phase III**: “Deconstruct” the assay: decide which parameters, when altered, significantly vary assay outcome.

Streptavidin: Biotin-Fl Assay Principle

Streptavidin (SA) is a tetravalent 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).

Assay Set-up

- PBS, PBS+0.1%BSA, and PBS+0.1% glycerol
- Add 25 µL of biotin-4-fluorescein (Fl) to black 96w plate
- Add 25 µL of inhibitor
- Add 25 µL of streptavidin (SA)
- Incubate for 30 min at room temperature
- Read fluorescence: Ex = 485 nm and Em = 515 nm

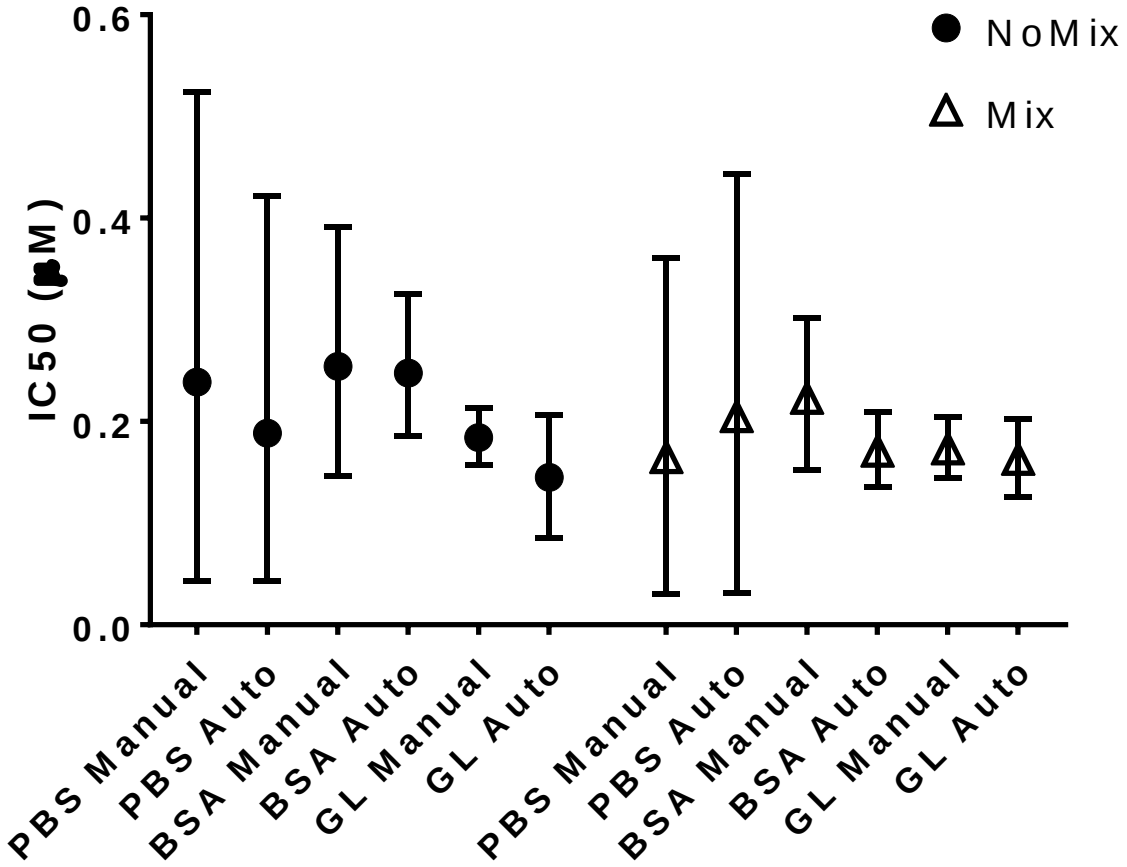


1. Waner, MJ; Mascotti DP. *Journal of Biochemical and Biophysical Methods* 70(6), 2008, 873-877.
2. Ebner, A; Marek, M; Kaiser, K; Kada, G; Hahn, CD; Lackner, B; Gruber, HJ. *Methods in Molecular Biology*, 418, 2008, 73-88.

Assay Development Parameters Evaluated

Parameter	Manual
Solution stability	X
Plate type	X
Background buffer	X
Inhibitor	X
Inhibitor conc.	X
Incubation time	X
Mixing	X
Pipette calibration	
Asp/Disp rates	
Fluid exit rate	
Air gaps	

Effect of Mixing on Assay Variability



Mixing was conducted by aspirating and dispensing 3 cycles after the third reagent was added. The plates were then incubated for 30 minutes at room temperature.

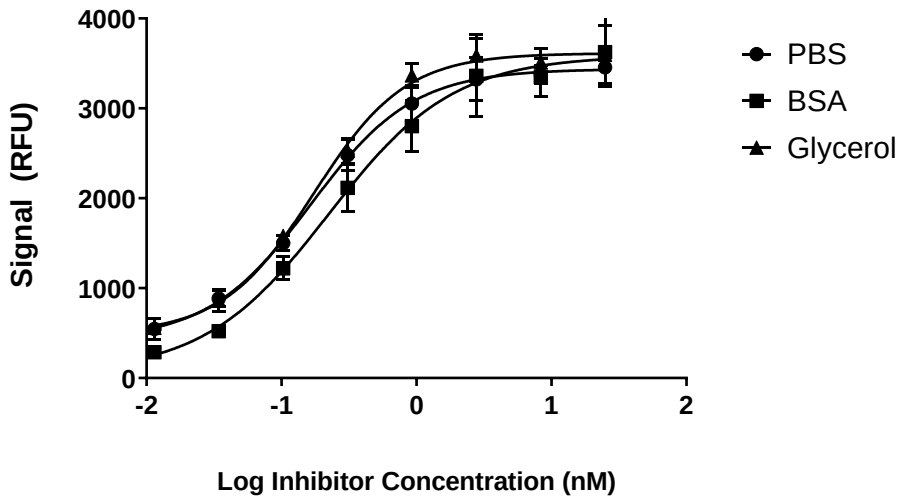
Key Takeaway

Buffer has an impact on mixing; Mixing improved BSA and glycerol buffers

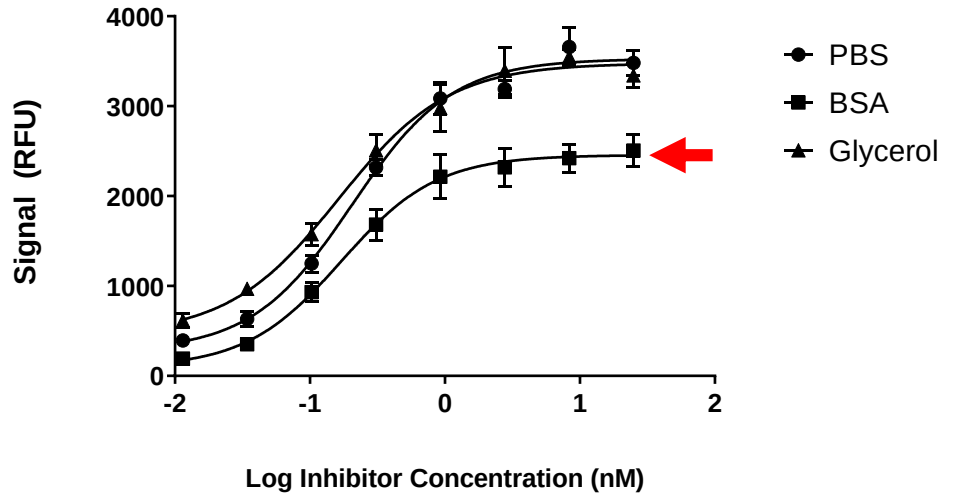
PBS = phosphate buffered saline / **BSA** = PBS + 0.1% bovine serum albumin / **GL** = PBS + 0.1% glycerol

Effect of Buffer Type on Assay Performance

Manual Mixing



Auto Mixing



Key Takeaway

Manual mixing can behave differently than automated mixing, especially depending on assay buffer ingredients.

Effect of Source Plates on Automated Liquid Handler Verification

A

B

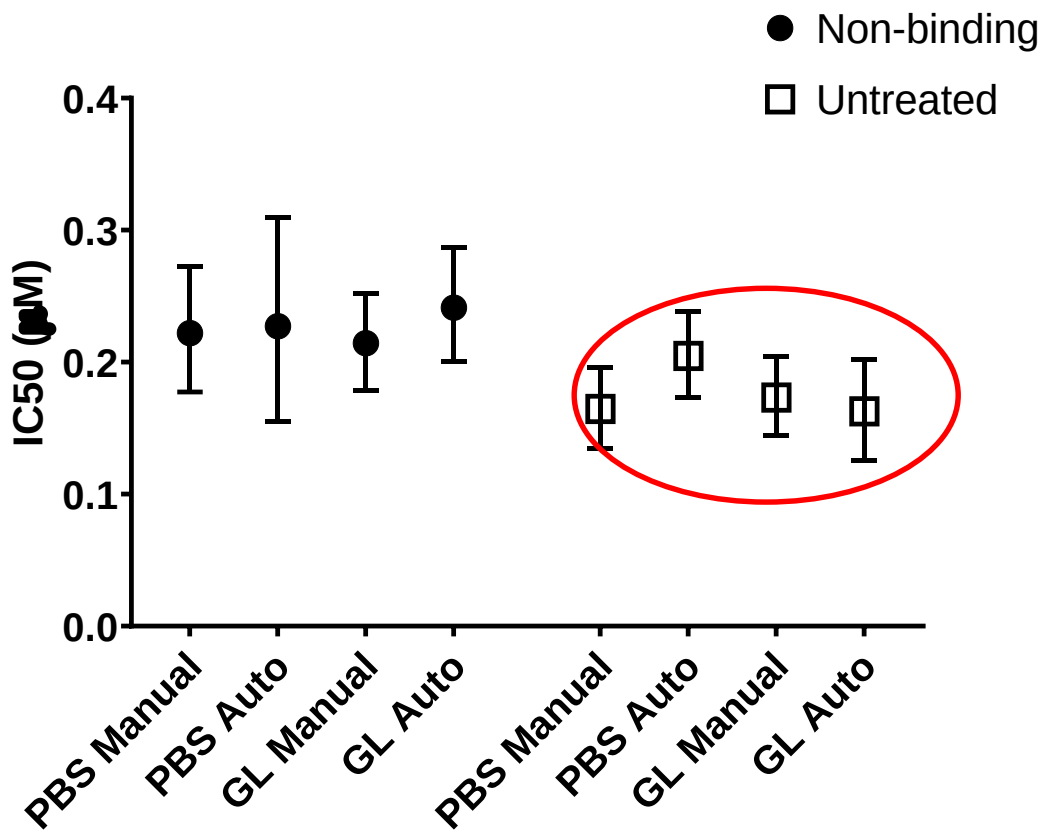
Artel MVS plates containing reagent from non-binding (A) and untreated (B) 96w black source plates.

MVS precision: 6.3% for non-binding and 1.2% for untreated

Key Takeaway

Source plate affected the destination plate – observed by visual inspection

Effect of Plate Type on Assay Variability



Black, 96-well plates used:

- *Corning #3650 (Non-binding)*
- *Corning #3915 (Untreated)*

Key Takeaway

When subjected to the assay, the uncoated plate yielded slightly lower variability.

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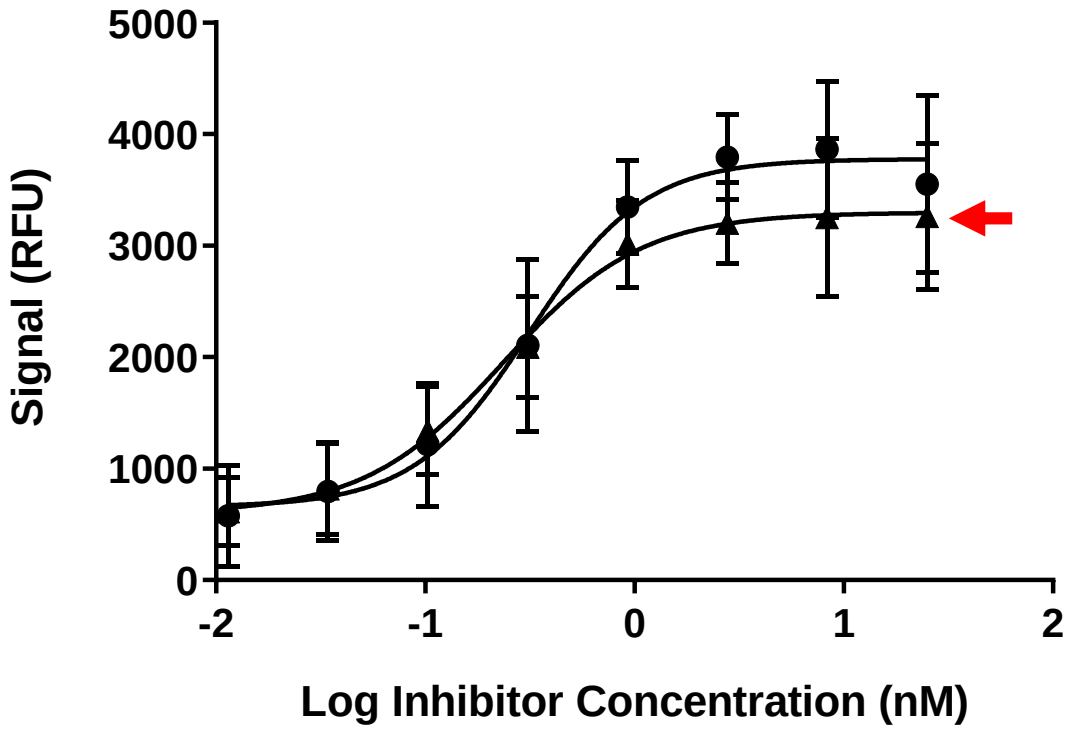
Effect of Reagent Temperature on Assay Variability



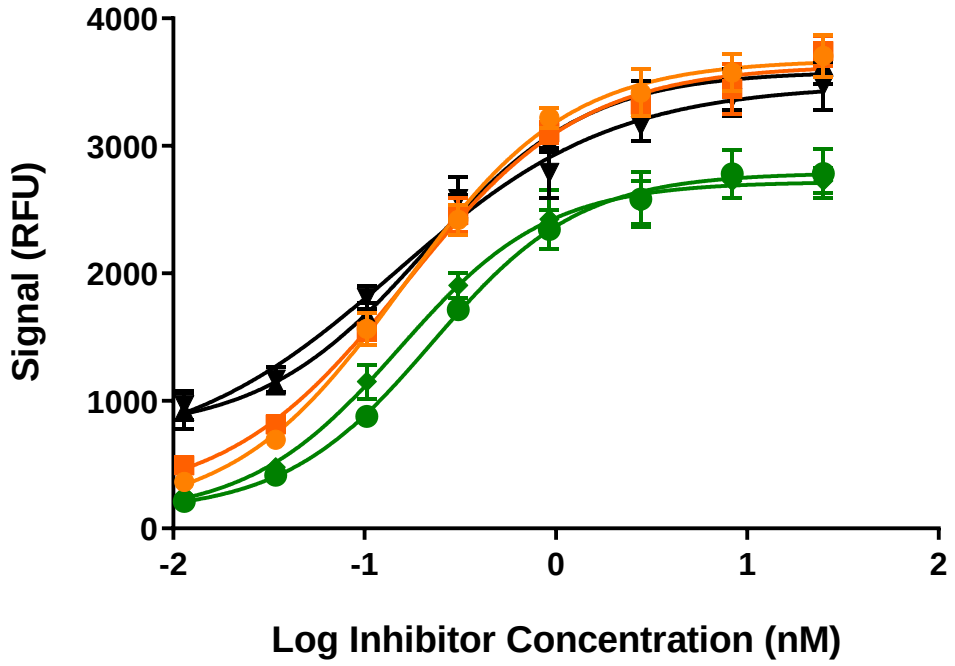
	IC50 (µM)
Cold Reagent Dispense	0.311
Room Temp Dispense	0.235

Key Takeaway

Cold reagent dispense yielded slightly lower potency

"Cold reagents" were stored at 4°C until use. The reagents were not maintained at 4°C during pipetting.

Effect of Aspirate (ASP) Speed on Assay Performance



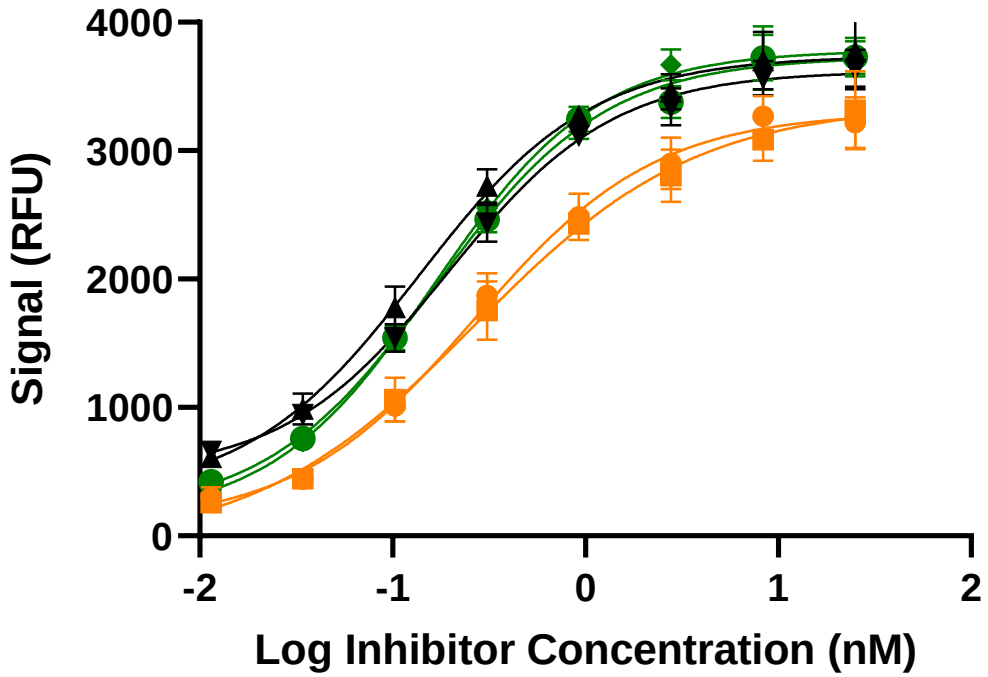
	IC50 (µM)
Aspirate 1 PBS	0.157
Aspirate 5 PBS	0.163
Aspirate 1 GL	0.151
Aspirate 5 GL	0.225
Aspirate 1 BSA	0.145
Aspirate 5 BSA	0.207

Key Takeaway

Aspirate speed affected potency for glycerol and BSA buffers

PBS = phosphate buffered saline / **BSA** = PBS + 0.1% bovine serum albumin / **GL** = PBS + 0.1% glycerol

Effect of Dispense (DISP) Speed on Assay Performance



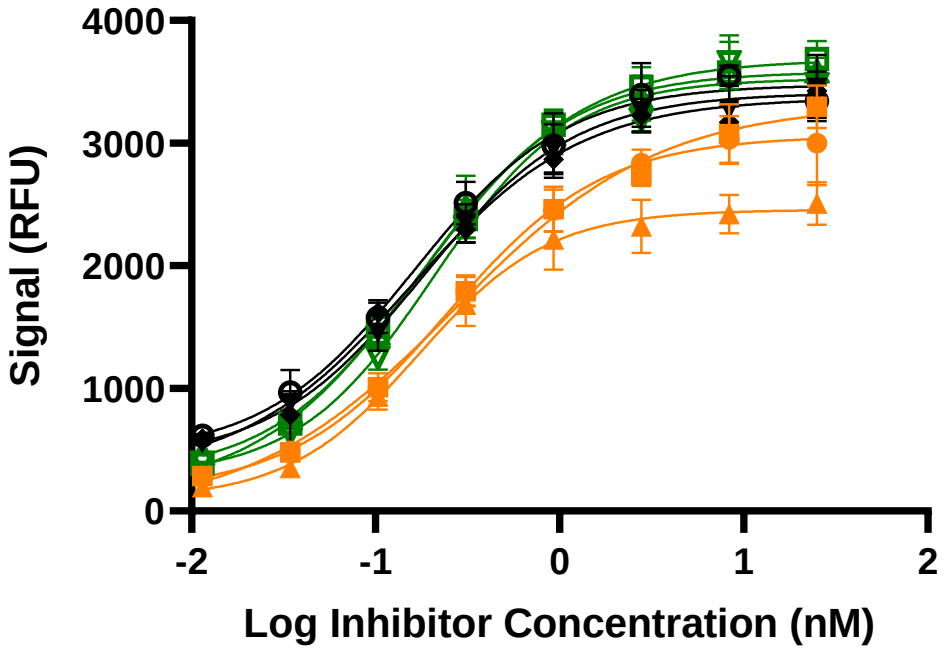
	IC50 (µM)
Dispense 1 BSA	0.268
Dispense 5 BSA	0.266
Dispense 1 GL	0.136
Dispense 5 GL	0.197
Dispense 1 PBS	0.164
Dispense 5 PBS	0.167

Key Takeaway

Dispense speed affected potency for glycerol but not PBS or BSA

PBS = phosphate buffered saline / **BSA** = PBS + 0.1% bovine serum albumin / **GL** = PBS + 0.1% glycerol

Effect of Air Gap on Inhibitor Potency



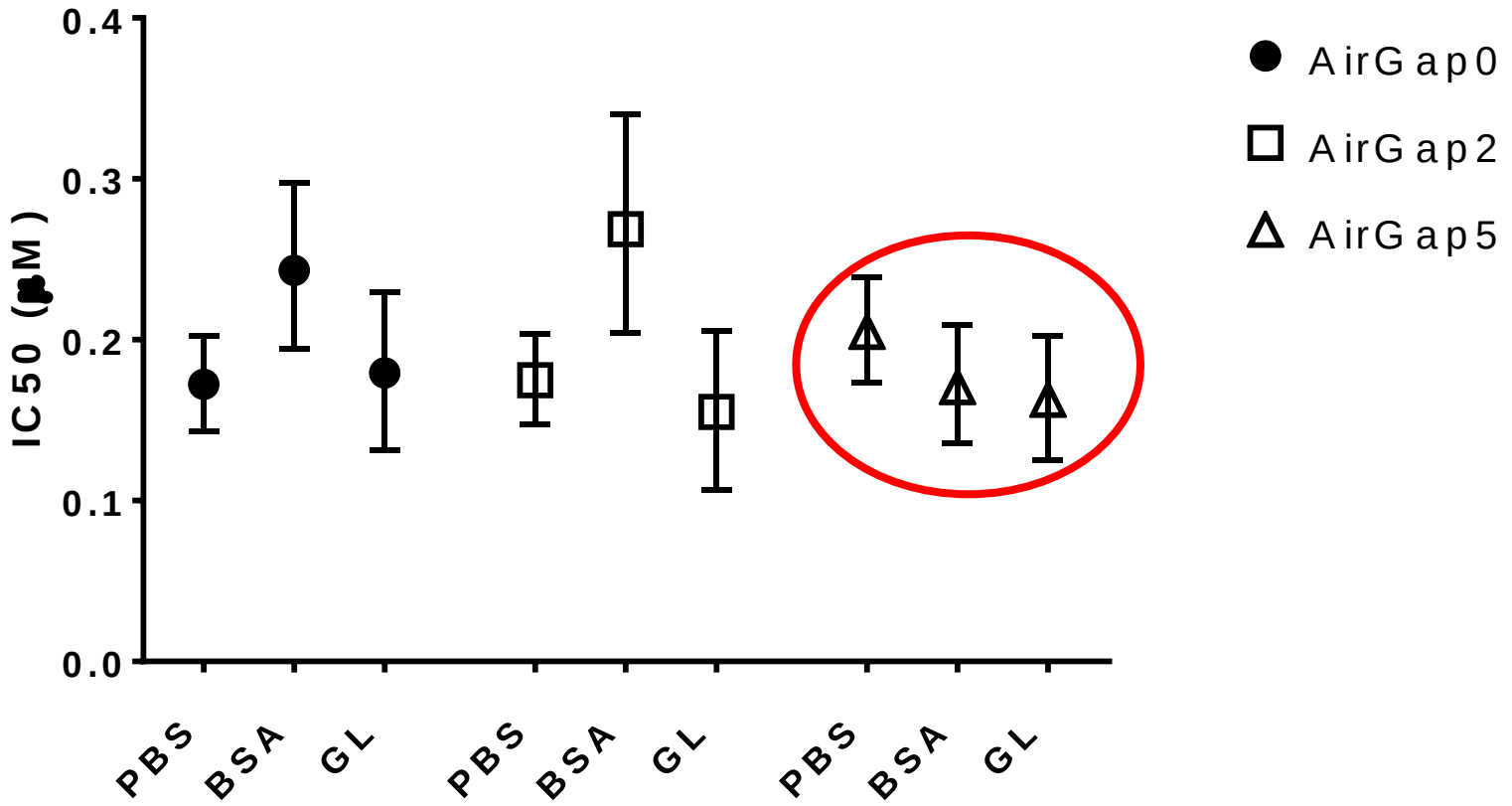
	IC50 (µM)
AirGap0BSA	0.243
AirGap2BSA	0.269
AirGap5BSA	0.171 ←
AirGap0GL	0.179
AirGap2GL	0.155
AirGap5GL	0.163
AirGap0PBS	0.172
AirGap2PBS	0.175
AirGap5PBS	0.205 ←

Key Takeaway

Air gap affects the assay containing PBS and BSA buffers with respect to potency and variability

PBS = phosphate buffered saline / **BSA** = PBS + 0.1% bovine serum albumin / **GL** = PBS + 0.1% glycerol

Effect of Air Gap on Assay *Variability*



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Study Summary

- Four assay parameters were identified which required optimization both during development at bench-scale ***and*** on the ALH.
  - Assay buffer selection
  - Mixing
  - Reagent stability
  - Plate type
- Certain ALH parameters were dependent on buffer type. Not all ALH parameters will effect an assay.

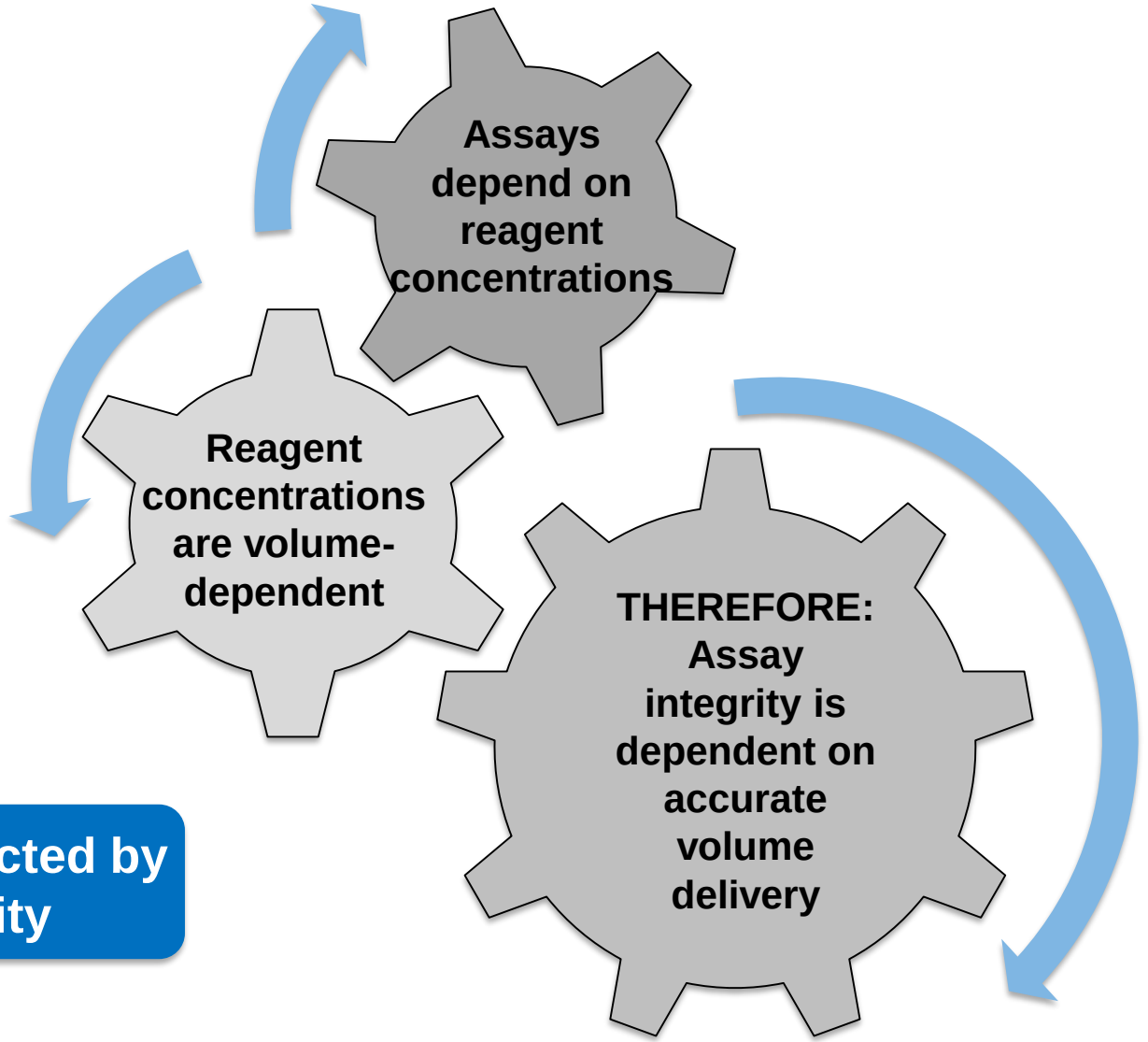
Conclusions

- Performing assay optimization ***AND*** liquid handler optimization ***together as a whole process*** reduces potential for error introduction and prolonged/difficult method transfer.

- Evaluate critical liquid handling parameters and potential sources of variability at bench scale and on the ALH for ***each new assay***.

Assay optimization *or* LH qualification alone isn't enough.

Putting It Together…



Assay results are impacted by liquid handling variability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Trust Your Results