Getting the Best out of Your Automated Liquid Handler

Nathaniel Hentz, PhD
On behalf of Analis Scientific Instruments

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Contact: nhentz@artel.co

Today's Agenda

- Understanding your automated liquid handler (ALH)
- Linking assay performance to ALH performance
- ALH measurement and accompanying applications
  - ALH liquid class optimization
  - Mixing & plate washing efficiency
  - Tip evaluation
  - Training
- Brief Artel technology description

Why We're Here Today

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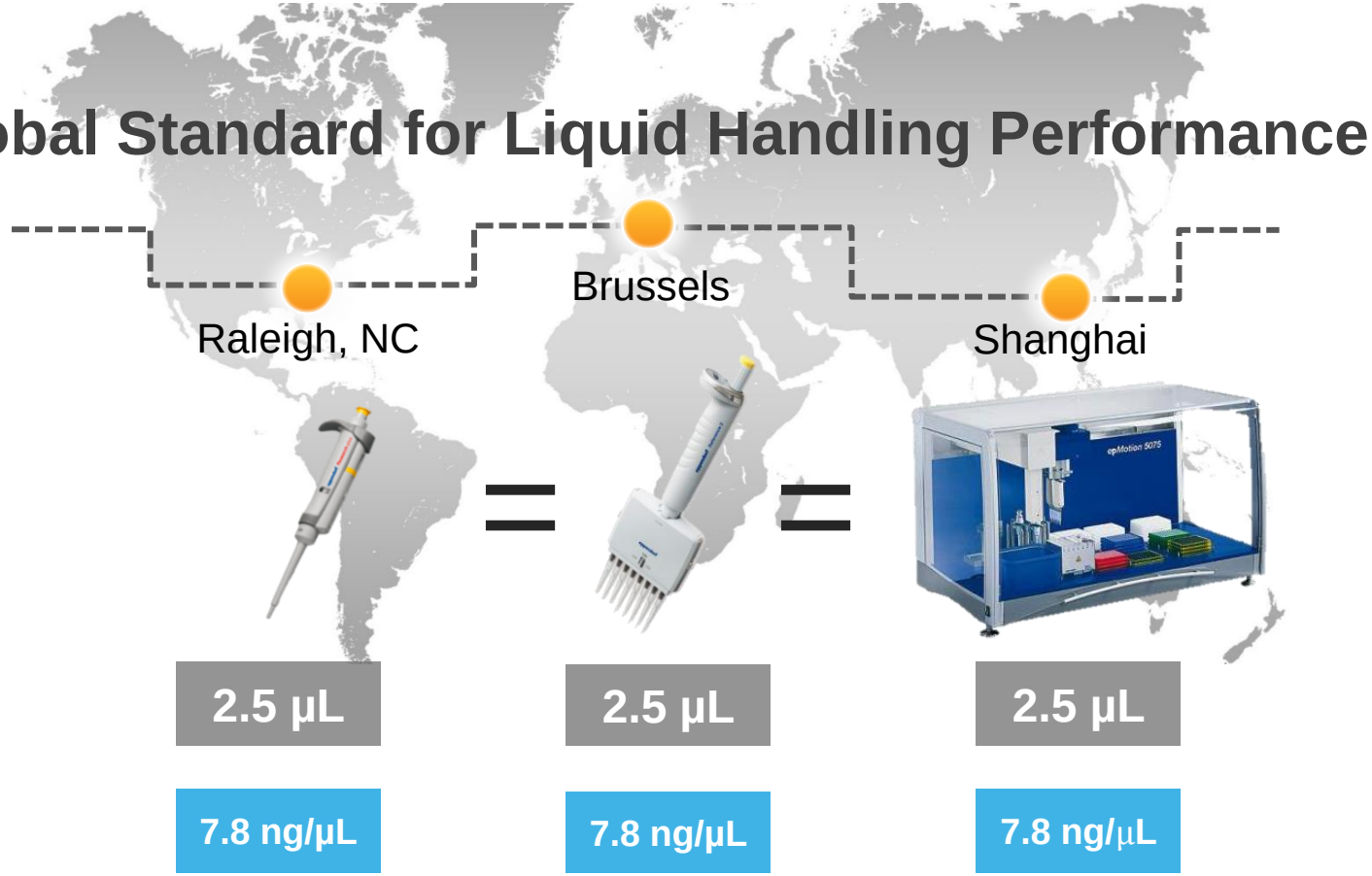


Understanding What Automated Liquid Handlers Can Do

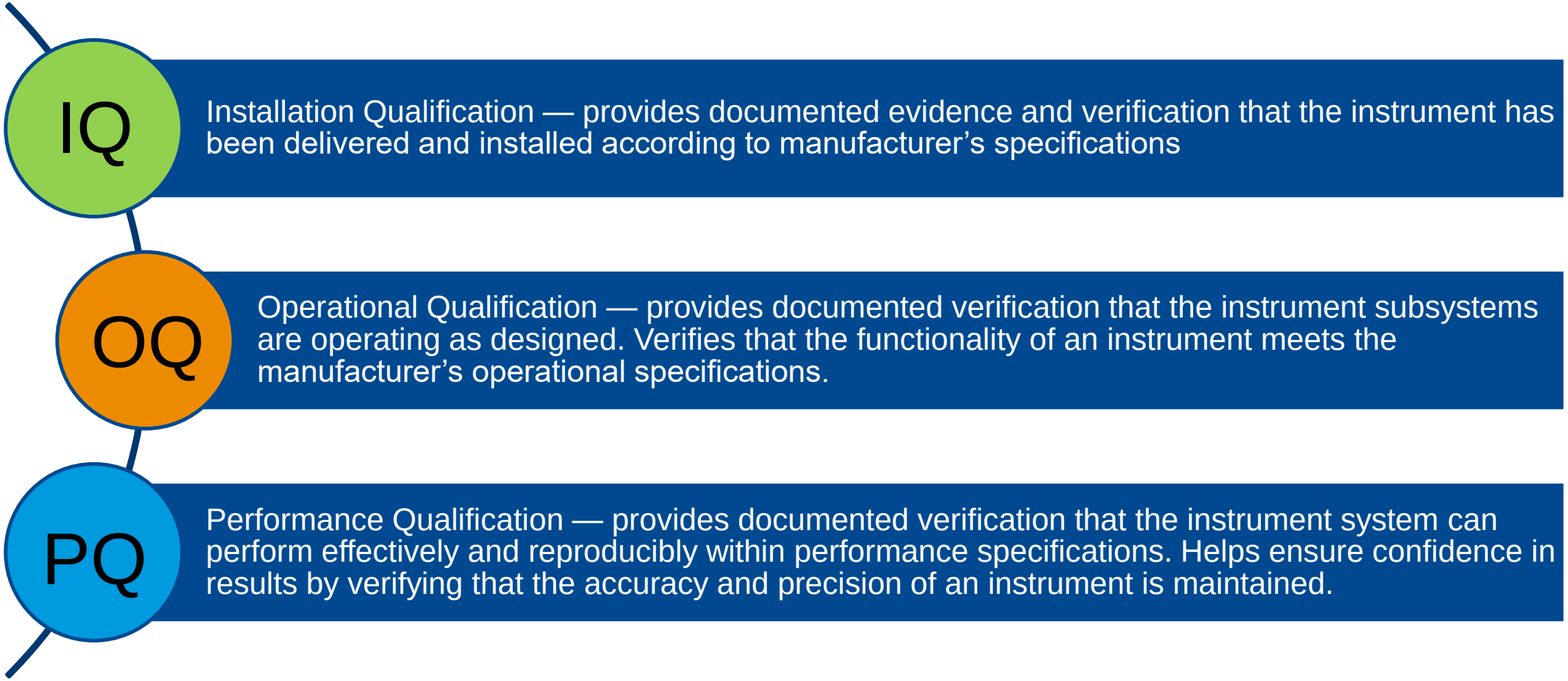
Multipurpose automated liquid handling workstations are designed to do any or all assay workflow operations including sampling, heating, cooling, filtering, mixing, washing, diluting and combining of reagents.

- Advantages: minimize contamination, free up lab personnel, reduce assay variability.

- Automated liquid handlers are NOT self-programmable, capable of on-the-fly adjustments, nor are they foolproof.

Automated liquid handling systems must be optimized for each assay

Setting up an Automated Liquid Handler



However…Many Things Affect Liquid Transfer

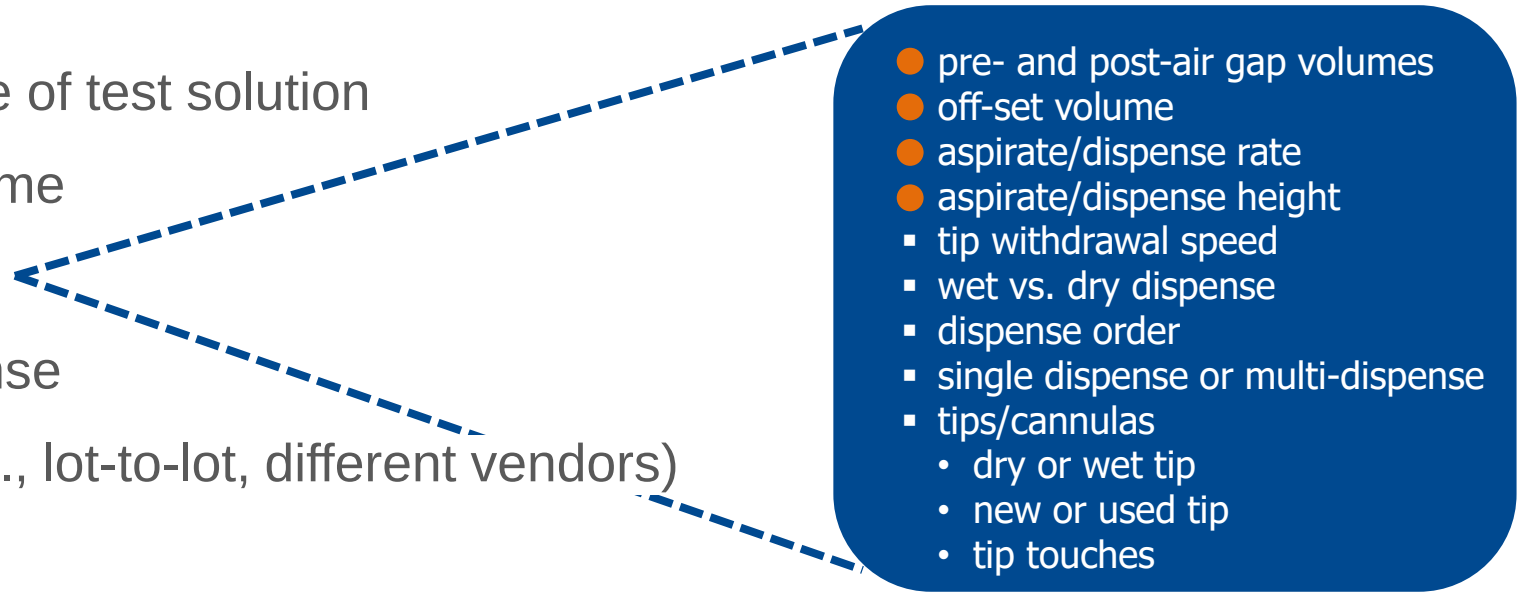
- Liquid properties (e.g., density, viscosity, surface tension, vapor pressure)
- Liquid transfer device
  - Components (e.g., tips, tubes, reservoirs)
  - Dispense principle (e.g., air/piston displacement, acoustic, pressure, piezo, etc.)
  - Liquid class settings or technique (e.g., air gap, asp/disp speed, immersion depth, etc.)
- Temperature

Performance Capabilities

Tip	Volume (µL)	Inaccuracy (±%)	CV (≤%)
X500	500	1	1
X500	250	3	3
X500	50	5	5
X50	50	1	1
X50	25	3	3
X50	5	5	5

Key to Liquid Class Optimization

- Fluid properties of master mix solutions in various NGS steps are not the same as DNA solutions or diluents!
- Rapid liquid class development for your automated liquid handler
  - Begin by selecting your ALH's default liquid class, such as "PCR", "viscous", "glycerol", etc.
  - Dispense desired volume of test solution
  - Measure dispensed volume
  - Optimize default settings
- Evaluate cold reagent dispense
- Evaluate disposable tips (e.g., lot-to-lot, different vendors)



Using MVS for Liquid Class Optimization

Before

Default ALH Liquid Class

STATISTICS

Target Volume (µL)	5
Mean volume for all channels (µL)	3.7170
Relative inaccuracy for all channels	-25.66%
Standard deviation for all channels (µL)	0.3907
Coefficient of variation (CV) for all channels	10.51%
Relative inaccuracy pass/fail limit	5%
Coefficient of variation pass/fail limit	5%
Status based on channel results	Failed
Status based on run statistics	Failed

After

Optimized ALH Liquid Class with MVS®

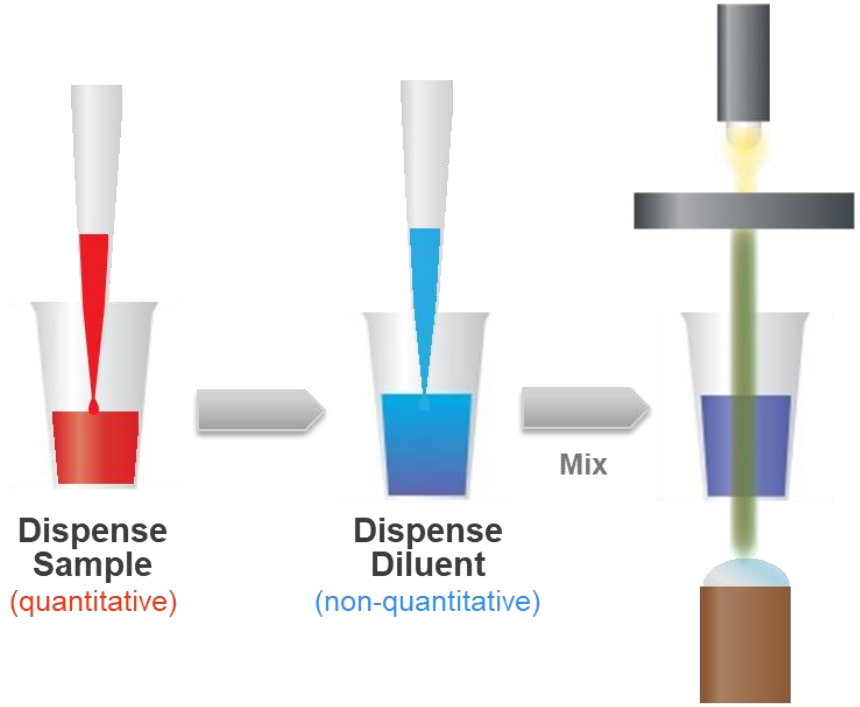
STATISTICS

Target Volume (µL)	5
Mean volume for all channels (µL)	4.9980
Relative inaccuracy for all channels	-0.04%
Standard deviation for all channels (µL)	0.0431
Coefficient of variation (CV) for all channels	0.86%
Relative inaccuracy pass/fail limit	5%
Coefficient of variation pass/fail limit	5%
Status based on channel results	Passed
Status based on run statistics	Passed

Default ALH liquid classes are not optimal for all assay solutions, yet it is so critical.

MVS Technology Description

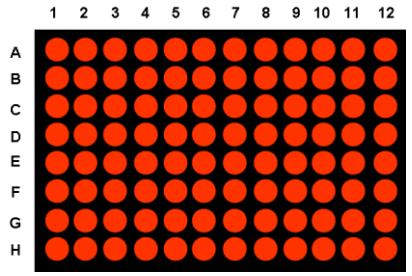
- Employs a dual-dye, dual-wavelength, ratiometric absorbance measurement for calculating the dispense volume.
- How it works: dyes of known concentration are dispensed into well-characterized microtiter plate. The plate is mixed on a plate shaker to ensure solution homogeneity. Absorbance readings are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



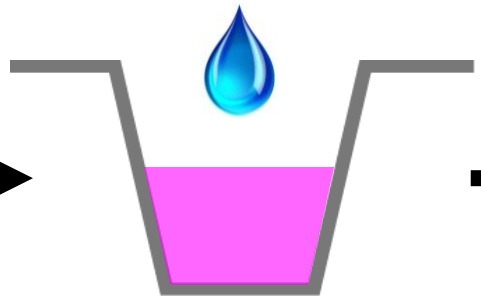
$$V_S = V_T \left(\frac{a_b}{a_r} \right) \left(\frac{A_{520}}{A_{730}} \right)$$

Basic Artel MVS Steps

Dispense red solution



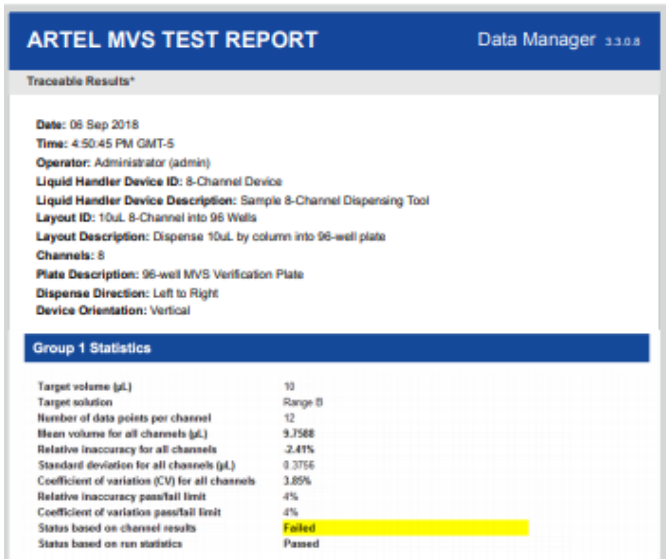
Add blue diluent



Shake on validated MVS plate shaker

Read plate

Report provides dispense accuracy *and* precision; statistics for entire plate, channel by channel, and dispense order.



ARTEL MVS TEST REPORT — Data Manager 3.3.0.8

Traceable Results*

Date: 06 Sep 2018
Time: 4:50:45 PM GMT-5
Operator: Administrator (admin)
Liquid Handler Device ID: 8-Channel Device
Liquid Handler Device Description: Sample 8-Channel Dispensing Tool
Layout ID: 10uL 8-Channel into 96 Wells
Layout Description: Dispense 10uL by column into 96-well plate
Channels: 8
Plate Description: 96-well MVS Verification Plate
Dispense Direction: Left to Right
Device Orientation: Vertical

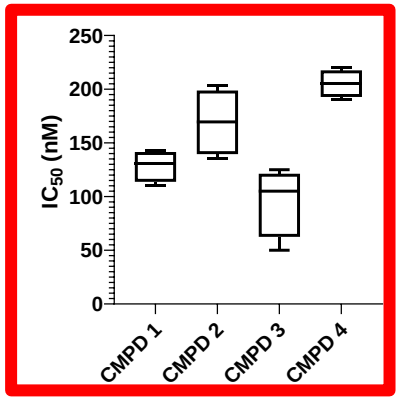
Group 1 Statistics

Target volume (µL)	10
Target solution	Range B
Number of data points per channel	12
Mean volume for all channels (µL)	9.7588
Relative inaccuracy for all channels	-2.41%
Standard deviation for all channels (µL)	0.3756
Coefficient of variation (CV) for all channels	3.85%
Relative inaccuracy pass/fail limit	4%
Coefficient of variation pass/fail limit	4%
Status based on channel results	Failed
Status based on run statistics	Passed

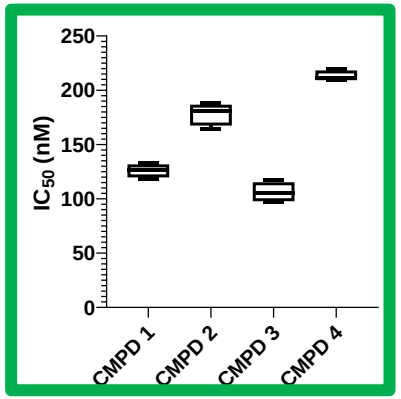
Data Quality is Dependent on Input!

Sample extraction
Sample dilution
Standard dilution
Liquid handling
Incubation
Mixing
Washing
Detection
Reagents
Tips
Plates
Plate seals

Assay



or



Sample/standard preparation Assay assembly steps Consumables

How Do We Employ MVS for an Assay?

Typical ELISA protocol:

1. Coat plate with 200 µL of Capture antibody, incubate overnight at 4ºC
2. Wash 4x with 200 µL of Wash buffer
3. Block plate with 300 µL of Block solution for 2 h at 37ºC
4. Wash 4x with 300 µL of Wash buffer
5. Add 100 µL of sample, prediluted 1:100 in Block solution
    a. Seal plate
    b. Incubate 1 h at 37ºC
6. Wash 4x with 300 µL of Wash buffer
7. Dilute Primary antibody 1:100 with Block solution
    a. Add 100 µL Primary antibody per well
    b. Incubate 1 h at 37ºC
8. Wash 4x with 300 µL of Wash buffer
9. Dilute Detection antibody 1:1000 with Block solution
    a. Add 100 µL of Secondary antibody per well
    b. Incubate 30 min at 37ºC
10. Wash 4x with 300 µL of Wash buffer
11. Add 100 µL of TMB Substrate per well
    a. Incubate 10 min at 37ºC
12. Add 100 µL of Stop solution per well
13. Read on plate reader at absorbance 450 nm

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Wash verification

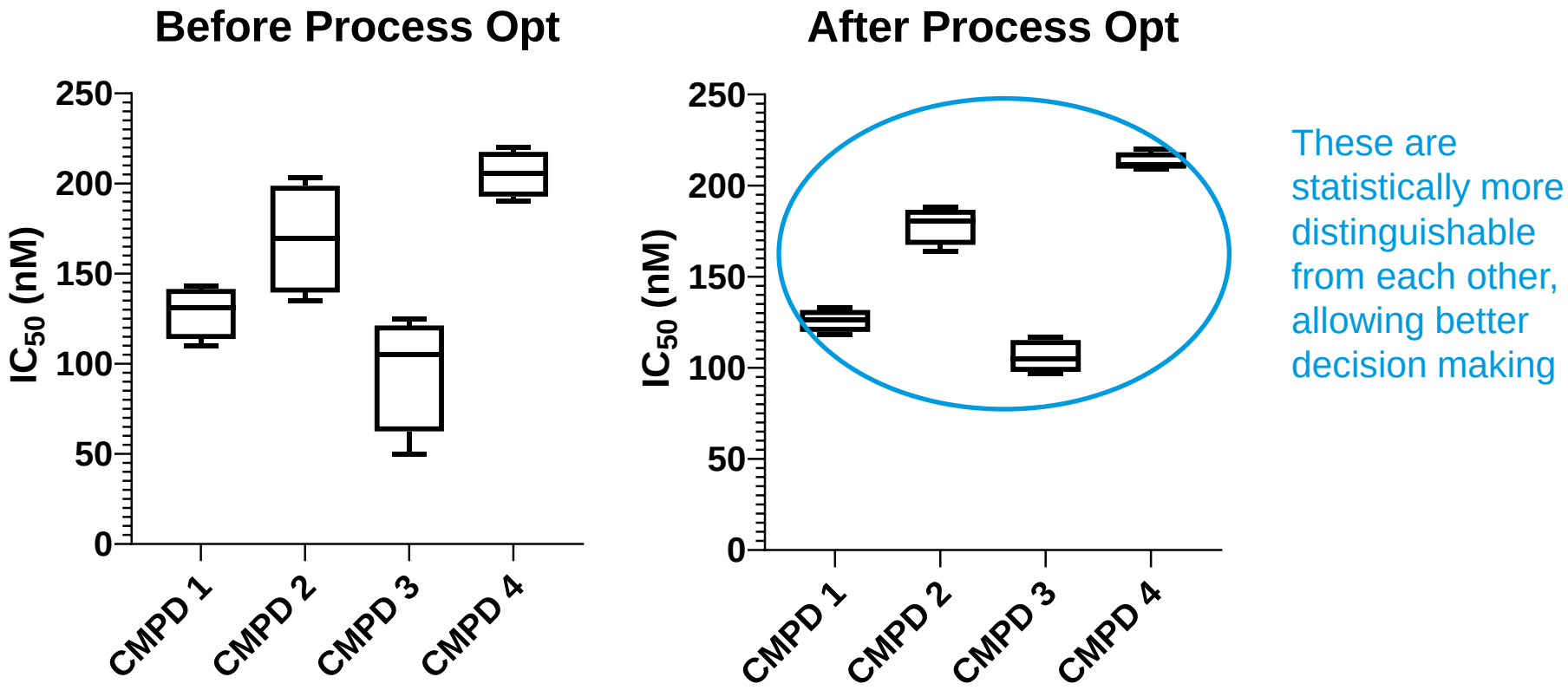
Volume verification

Serial dilution accuracy

Mixing verification

Liquid class optimization

Optimizing the Assay Workflow



These are statistically more distinguishable from each other, allowing better decision making

CMPD1, 2, 3, and 4 are structurally related compounds with similar potencies (n=4)

Thank You!

Let’s take some questions now

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