Evaluating Disposable Pipette Tip Performance: Guided by Science

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On behalf of Analis

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Today's Agenda

Introduction

- optimizing a workflow process
- sourcing disposable pipette tips

Why are pipette tips so variable?

- manufacturing
- fluidic properties

Artel MVS can help with a pipette tip verification program

- example methodology
- example data analysis

Summary of benefits and discussion

Artel MVS: Optimizing Your Automated Workflow

Artel MVS can help automation labs optimize many parts of an assay process. This is important when transferring a method or developing a new method.

Artel MVS: Optimizing Your Automated Workflow

Today's seminar will focus on one piece: verifying performance of disposable pipette tips

Pipette Tip Shortage

"Labs across the country are facing backlogs in coronavirus testing thanks in part to a shortage of tiny pieces of tapered plastic.

Researchers need these little disposables, called pipette tips, to quickly and precisely move liquid between vials as they process the tests.

As the number of known coronavirus cases in the United States passes 4 million, these new shortages of pipette tips and other lab supplies are once again stymieing efforts to track and curb the spread of disease." *K.J. Wu, Wall Street Journal, Published July 23, 2020 / Updated Aug. 15, 2020*

Key facts

- **Manufacturers are ramping up capacity**
- **Labs are going to alternate sources**
- **Labs are re-using tips**
- **Quality is critical**

Customers Have Options

Switch tip manufacturers

- Differences in manufacturing quality
- Differences in raw materials
- Impact on volume accuracy and precision

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Clean & re-use

- Effect of cleaning on volume delivery
- Tip re-use and structural integrity
- Build-up of cleaning agent on tip

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Buy larger inventory

- Multiple lots of tips
- Tip quality assurance of manufacturing ramp-up
- Tip storage / tip quality

Pipette Tip Verification: Where it Matters

- Clinical diagnostics labs running automation
- Clinical genetics testing labs
- Drug testing labs
- CROs/CDMOs/CMOs
- Drug discovery
- Manufacturing QC labs > analytical method transfer, routine QC
- Analytical labs supporting clinical and process development

Labs where quality matters, e.g., patients are involved, quality differentiates from competitors, important process decisions are being made, regulatory requirements

Many Things Affect Liquid Transfer

- **The liquid properties**
- The liquid transfer device
  - Components
  - Dispense principle
  - Liquid class settings or technique
- The temperature

Liquid Properties and Their Effects on Dispense

- **Density** – weight per volume (think NaCl solution)
  - The “heavier” a solution is, the more drag on the air space causing less volume to be dispensed; *Adjust volume with reference to water*

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  - High vapor pressure solutions evaporate and as a result force liquid out the orifice; *Pre-wet the tip several times to minimize effect*

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  - Viscous solutions leave film on walls of tip, thereby reducing dispense volume; *Reduce pipetting speed, use wide orifice tips, or use reverse pipetting technique along with volume adjustment*

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- **Surface Tension** – strength with which intermolecular forces hold a fluid together (think detergent)
  - Fluids with reduced surface tension tend to leave a film on the walls of the tip or induce bubbling; *Reduce pipetting speed or use reverse pipetting technique*

Many Things Affect Liquid Transfer

- The liquid properties
- The liquid transfer device
  - **Components**
  - Dispense principle
  - Liquid class settings or technique
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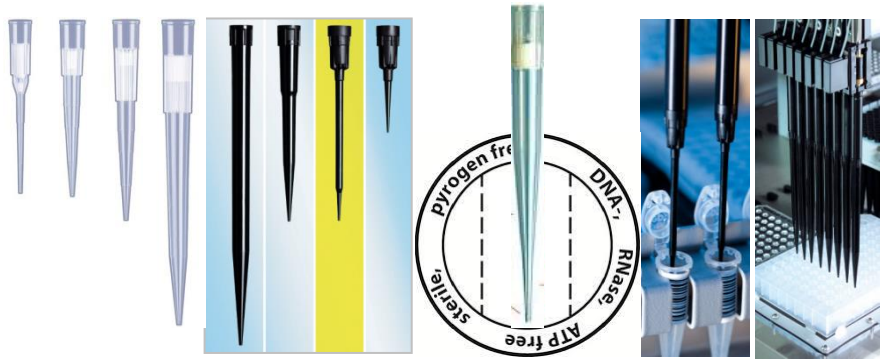
Pipette Tips are NOT Created Equal

Today, there are many things to consider when sourcing tips:

- Tip size and volume
- Tip type and material
- Manufacturer
- Cost
- Availability



ADVICE

- *Tip studies highly recommended when making this choice*
- *ALH manufacturers typically provide support only when using their recommend tips*

Many Tips to Choose From

- **Non-Barrier (Standard)** – designed for everyday lab work.
- **Barrier (Filter)** – if you will be pipetting something that could contaminate your pipette (e.g., volatile, corrosive, or viscous chemicals) or your samples (e.g., PCR). Most filters only slow the liquid from entering the pipette barrel.
- **Sterilized** – pre-sterilized tips work as designed; non-sterilized tips need to specifically say that they can be autoclaved. Not all plastics are equal, and tips can become deformed after an autoclave cycle.
- **Low Retention** – Tips are treated to become hydrophobic, which keeps liquid from sticking to the inside of the tips. Improves sample recovery when pipetting detergent containing solutions or other liquids with low surface tension.
- **Graduated** – Graduated tips have measurement markings on their side. If graduations are molded into the plastic, then liquids can cling.
- **Ergonomic** – specially designed ergonomic tips require lower insertion/ejection forces (to reduce the risk of RSI). These types of tips may not be good for repeated use.

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In some cases, customers may autoclave (sterilize) tips in-house

- *Standard autoclaving conditions are exposure to 121 °C, 2 bar for 20 minutes.*
- *Polypropylene becomes soft at approximately 110 - 120 °C, with a melting temperature of approximately 160 - 180 °C.*
- *Therefore, autoclaving according to standard method does stress the material.*

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Pipette Tip Fit

- Proper fit: There is no truly “universal fit” pipette tip. The right tip will install easily without applying a lot of force and will stay in place on the pipette. These are usually manufactured by the ALH vendor.
- Does it fit?: Chances are it’s the wrong tip fit if the tip ejector doesn’t function properly in releasing the tip. If the pipette seems to be in good working order, but liquid is leaking assume it may simply be the wrong tip.
- Sealing rings: Some ALHs use an O-ring on the pipette nozzle to create an air-tight seal between the tip and pipette, which help improve accuracy.

Pipette Tip Materials

- Stainless Steel – native or Teflon coated
- Polypropylene – virgin or recycled
  - Mold slip agents (e.g., oleamide, erucamide, or stearamide)
  - Biocides (e.g., DiHEMDA)
  - Residual plasticizers from PP manufacturing
  - Hydrophobic additives (e.g., silicone)
- Conductive – graphite infused polypropylene

Varies by manufacturer

These can affect both the assay biology and pipetting performance

Tip Purity Matters

- “Scientific research is composed of an iterative series of experiments in which the value of each experiment depends on the fidelity of the one that preceded it. Anything introduced along the way can alter the accuracy of the data can divert the experimentation onto a nonproductive path.”
- “The result: wasted weeks, if not months, of irreplaceable research time, effort and expense. Given this, it is critically important to have safeguards in place to prevent the introduction of a contaminant into the experimental reaction. Any impurity that can alter data fidelity can have disastrous consequences for a research project and the ability to publish scientific findings.”

Rainin Technical Report: Tip Purity Matters, 11/2018, Mettler Toledo

Pipette Tip Quality Certification Example

- Sartorius pipette tips are produced of non-recycled, virgin polypropylene, and the filters of polyethylene.
- During production of these materials the following agents are not used or intentionally added: slip agents (including oleamide, erucamide, stearamide), biocides (including di(2-hydroxyethyl) methyldodecylammonium salts (DIHEMDA), plasticizers (softeners/phthalates), silicone or latex.
- Sterility Pre-sterilized pipette tips are sterilized in accordance with *ISO 11137-1* and *ISO 11737-2*
- Purity Testing – free of DNase, RNase, human DNA and endotoxins (pyrogens) by an independent laboratory.
- Additional *Certificates of Purity* may be available: Trace metals, PCR ready (inhibitor free), ATP-free

Tip Quality

Good Tip

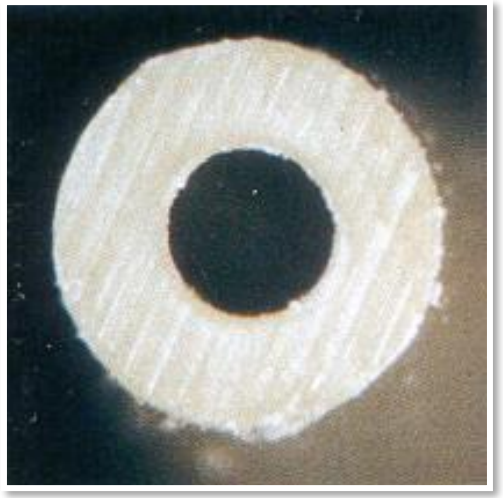


Photo By H. Ulrich

- Centered orifice
- Uniform appearance and finished looking
- Often made from higher grade materials

Bad Tip

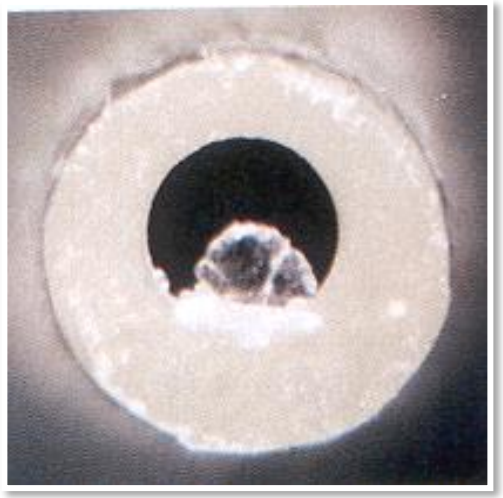


Photo By H. Ulrich

- Off-centered orifice
- Not as “finished” or “polished” looking
- Flashing material still present

Evaluate your tips by giving them a visual inspection

- Roll them on the table to see how straight they are.
- Does there appear to be any external inconsistencies or any irregularities in either cavity?

If the tips are not molded well, this will affect pipetting performance.

Designing a Tip Performance Verification Program

Measurement

- Assay
- Artel MVS technology

Workplan

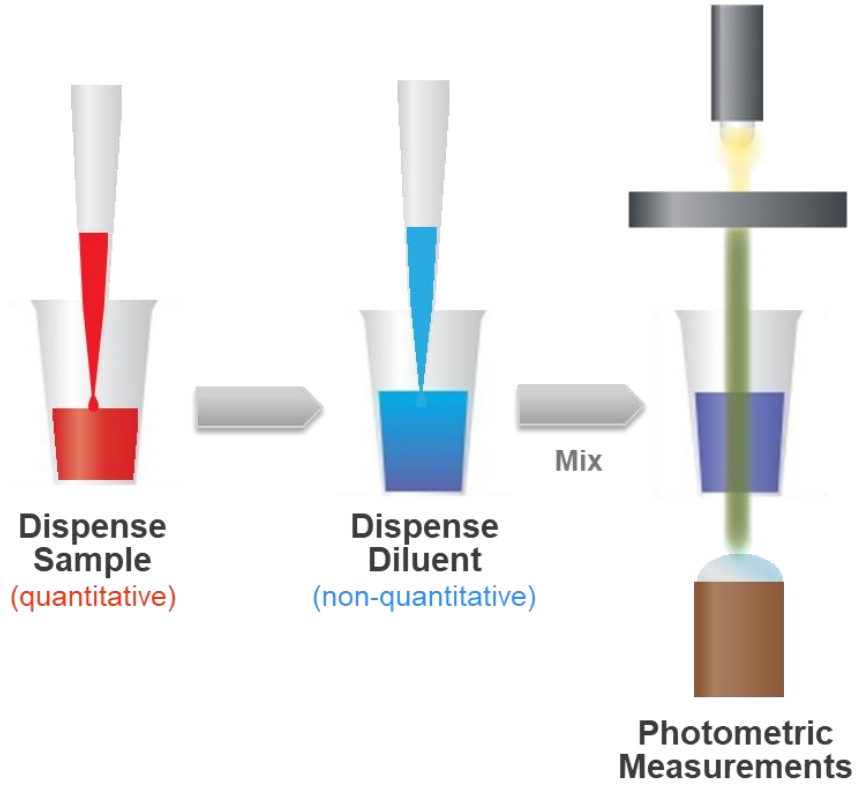
- Frequency
- Replicates
- Analysis tools

Specifications

- Based on data – both MVS and assay

How to Measure: MVS Technology Description

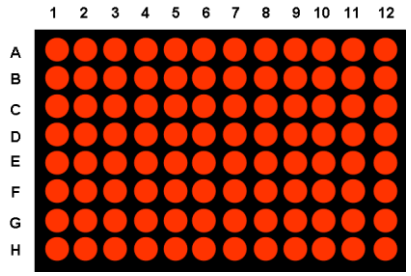
- Employs a dual-dye, dual-wavelength, ratiometric absorbance-based measurement method for calculating the dispense volume.
- Dyes of known concentration are dispensed into a well-characterized microtiter plate. The plate is mixed on a validated plate shaker to ensure solution homogeneity. Absorbance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



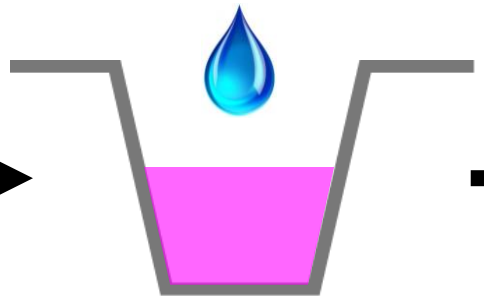
$$V_S = V_T \left(\frac{a_b}{a_r} \right) \left(\frac{A_{520}}{A_{730}} \right)$$

MVS Workflow

Dispense red solution



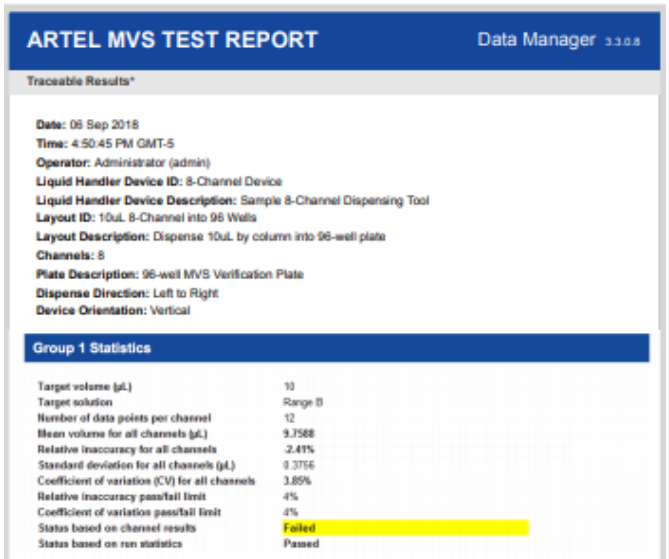
Add blue diluent



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

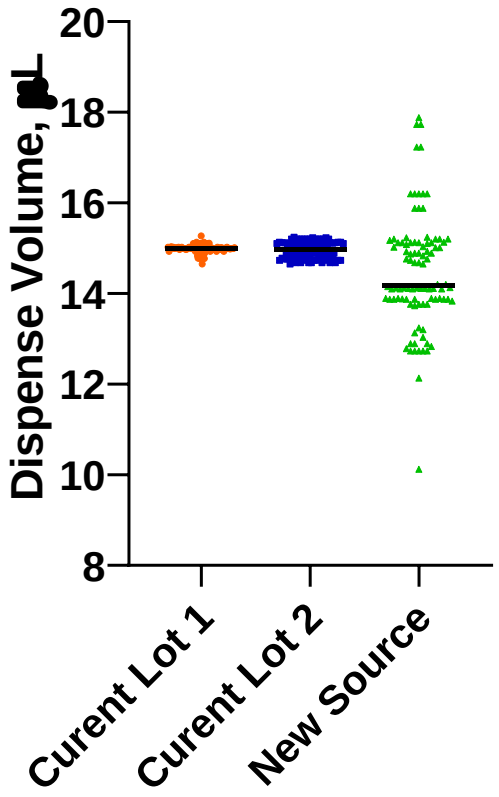
Traceable Results*

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

Disposable Pipette Tip Evaluation



Current Lot 1
Accuracy: 14.98 µL
Precision: 0.68%

Current Lot 2
Accuracy: 14.97 µL
Precision: 1.21%

New Source
Accuracy: 14.41 µL
Precision: 10.94%

Experimental Details
- Target volume = 15 µL
- Disposable tips from two lots currently used and a new source of universal tips
- N = 96
- Motivation: New source tips cost 30% less

Data Summary
- Lots 1 and 2 from current manufacturer compare favorably
- New tip source do not meet “pass” specifications

Guidance on Measuring Disposable Tip Performance

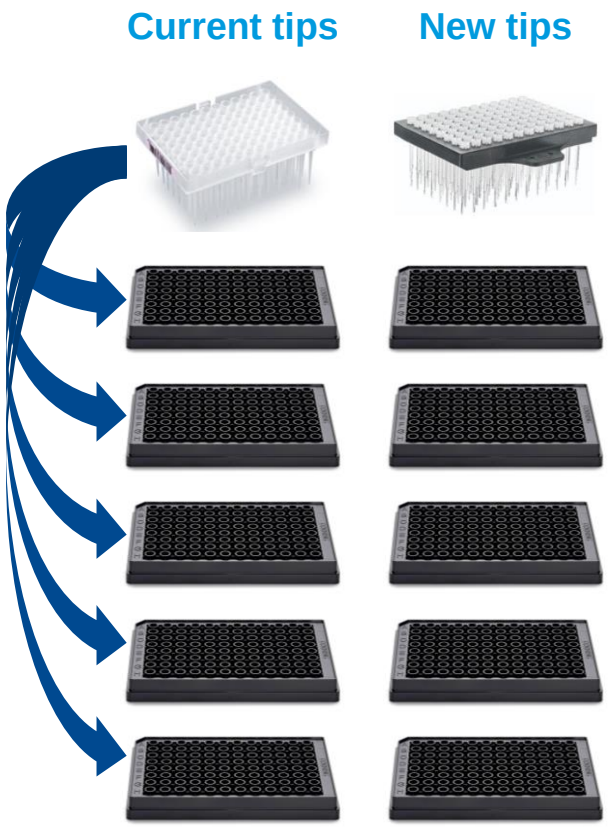
- Select correct tips to be used in automated assay; consider volume required and tip volume
- Identify how tips will be (or are) used in your automated assay
  - Single volume aspirate and dispense
  - Single aspiration and repeated dispense
  - Use once, discard
  - Repeated use

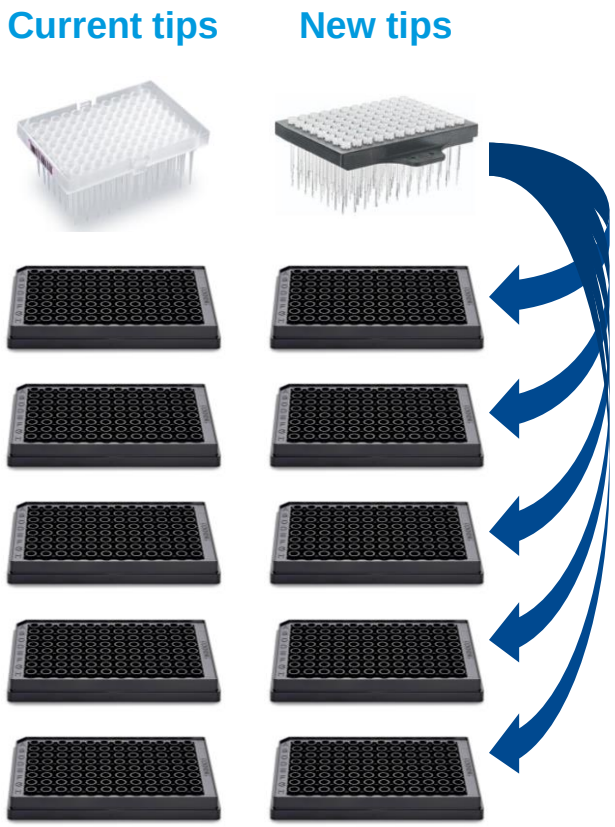
- Identify format of your automated liquid handler, e.g., 384-channel, 96-channel, 8-channel
- Determine whether multiple ALHs will use these tips
- If tips will be used for any number of assays and volumes, then you will want to evaluate high, medium, and low volumes for the tip
- Specify your pass/fail criteria!

Example: Dispensomatic ALH-96 Using P20 tips

2 µL dispense across five plates, same tips

5 replicates for each tip type at 10% nominal volume





20 µL dispense across five plates, same tips

5 replicates for each tip type at nominal volume

What to Look For? (Statistically Different or Experimentally Relevant)

Volume Accuracy and Precision

- Column
- Row
- Plate
- Overall replicates (n ≥ 3 plates)

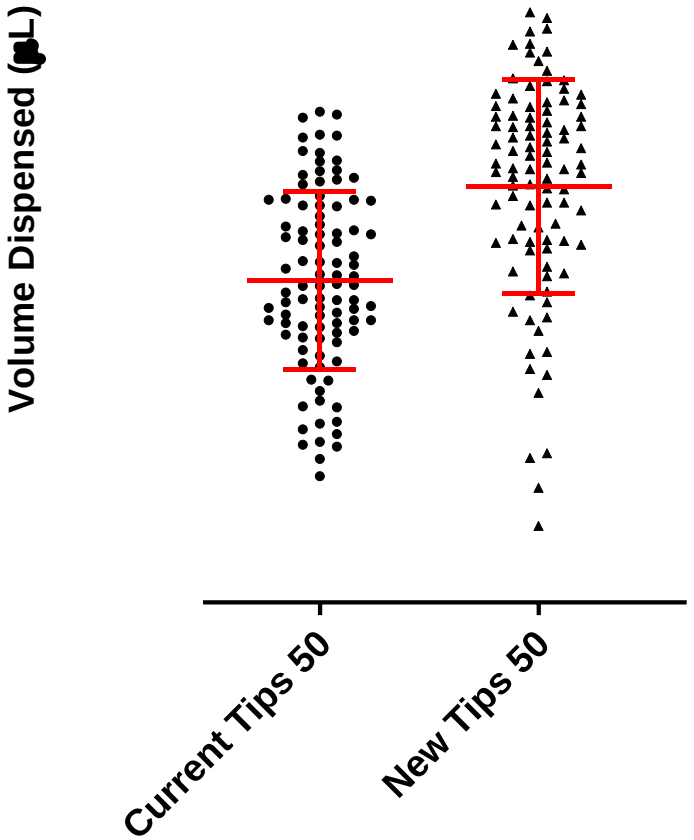
Trending / Patterns

- Plate to plate / tip to tip
- Lot to lot
- Number of re-uses

Analysis Methods

- *Traditional statistics, e.g., mean, standard deviation and %CV*
- *Statistical process control charts*
- *ANOVA or Welch's t-test to compare lots, tip manufacturers, or effect of washing/re-use*

Statistically Different vs Experimentally Relevant



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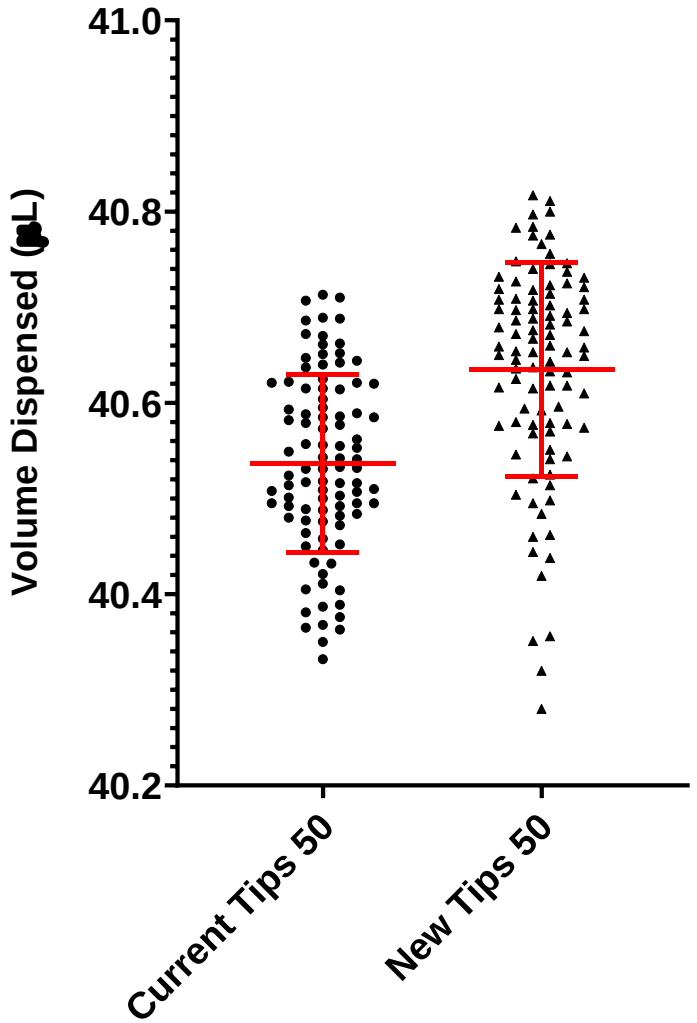


	Current Tips 50	New Tips 50
Number of values	96	96
Mean	40.54	40.64
Std. Deviation	0.09277	0.1117
Std. Error of Mean	0.009469	0.01140
Coefficient of variation	0.2289%	0.2748%

Welch's t-test (P < 0.05):

Means different? yes
Variance different? no

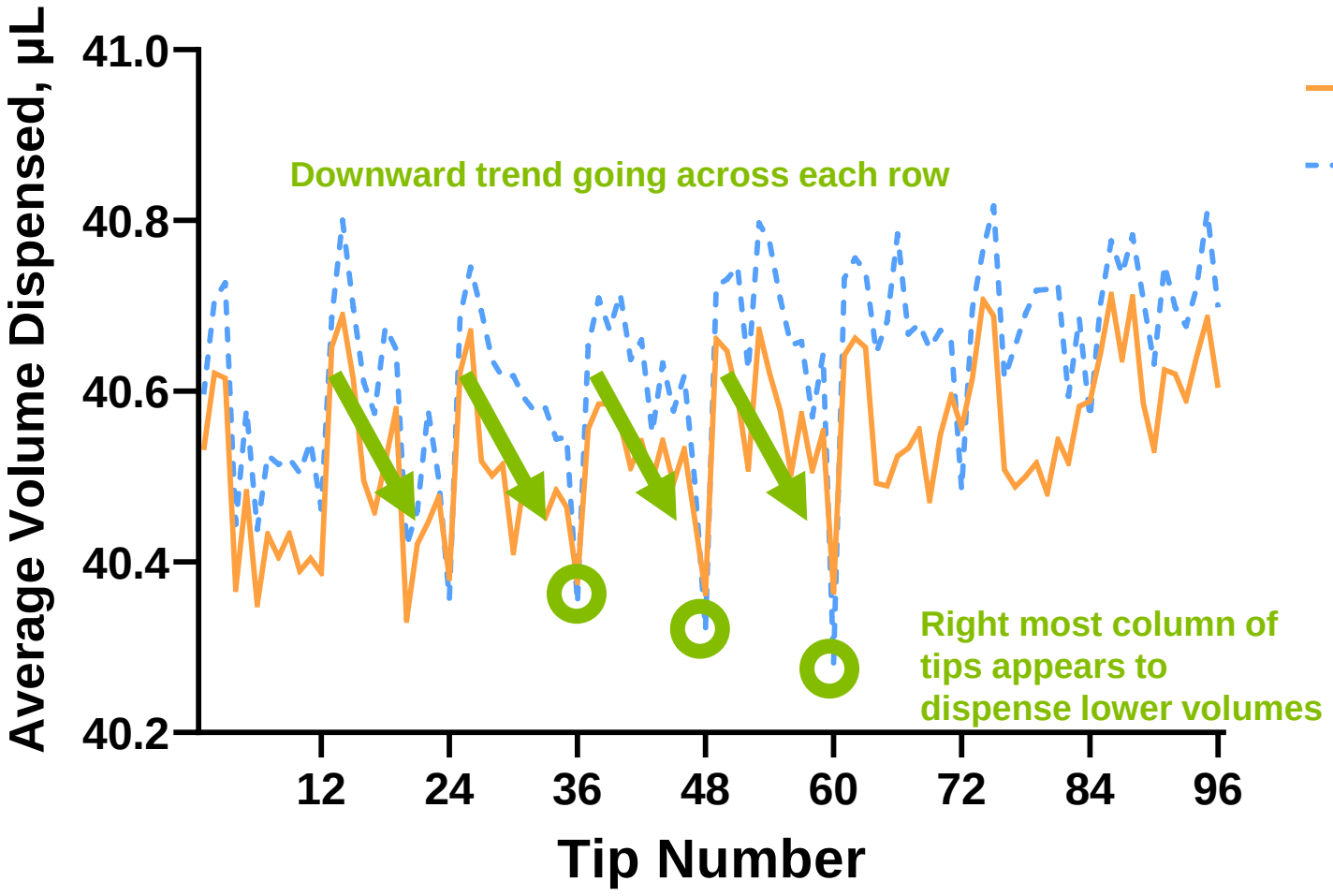
The data clearly shows a difference in means, but….it is only 0.1 µL

- Is this enough to warrant rejection?
- Advice to customer might be to re-calibrate ALH with new tips

The variability is slightly larger for the universal tips

- Is this enough to warrant rejection?
- Advice to customer might be to consider the current assay

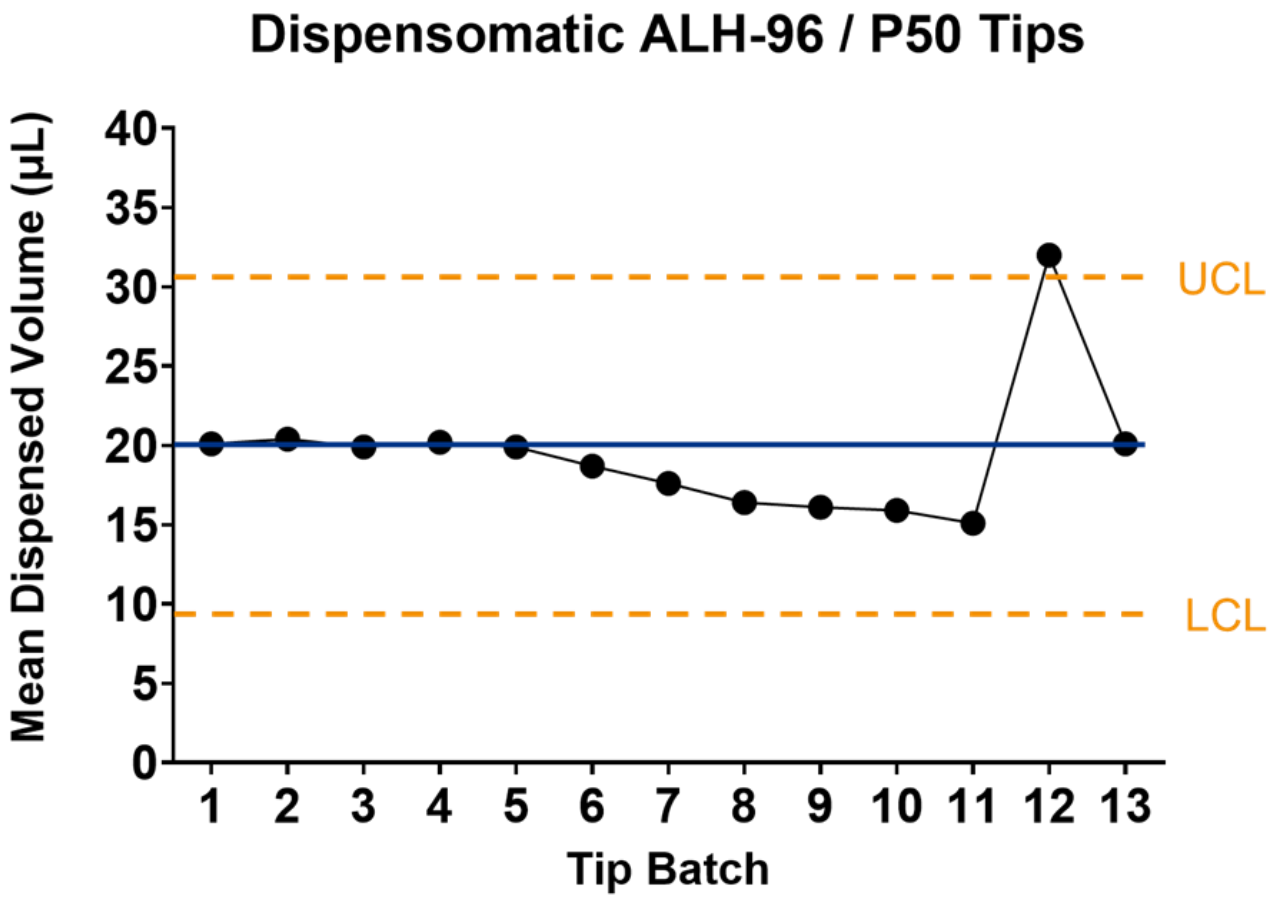
Short-Term Comparison: Scatter Plots



Experimental Details
- 40-µL dispense with 50-µL tips on ALH3
- 96-channel head
- Each data point is the average of 10 replicates

Data Summary
- New tips show the same pattern as the Current tips: row-by-row and entire plate
- The trends are likely due to the ALH and not the tips
- The New tips yield a higher average volume

Long-Term Trend Analysis: Statistical Process Control Chart



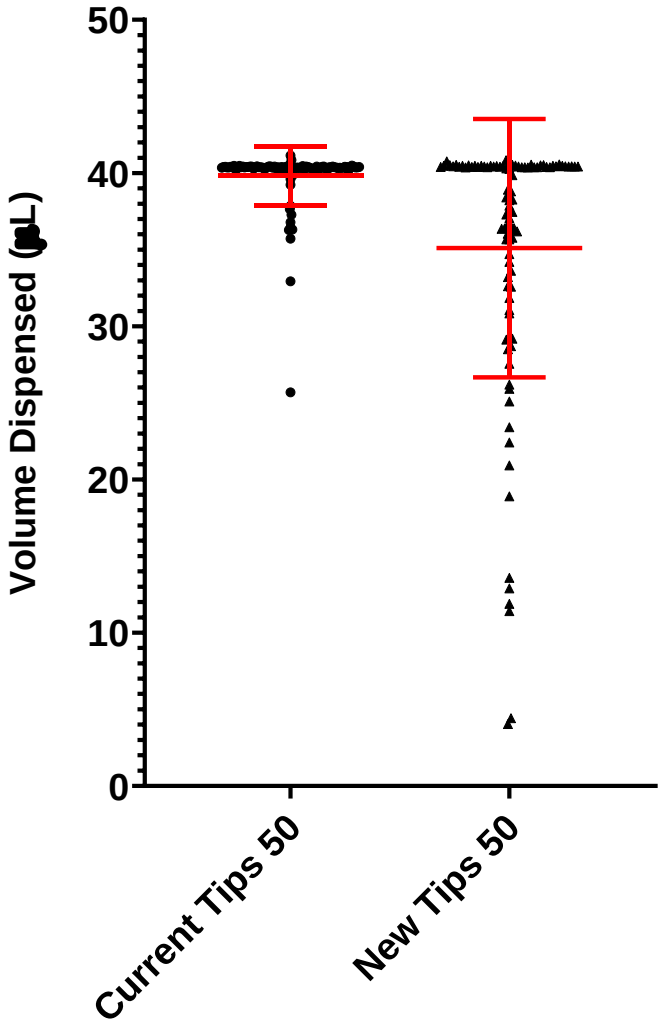
Experimental Details

- 20-µL dispense with 50-µL tips
- 96-channel head
- Each data point is the average of 96 replicates from a different tip lot

Data Summary

- Tip lots 1-11 and 13 are within the upper and lower control limits (UCL and LCL, respectively) bounds
- Tip lot 12 fell out of spec (Action Limit) and therefore should be discarded.
- Tip lots 6-11 are trending downward so warrant careful monitoring (Alert Limit)

Sub-Optimal ALH May be More Sensitive to Different Tip Sources



	Current Tips 50	New Tips 50
Number of values	96	96
Mean	39.81	35.10
Std. Deviation	1.924	8.435
Std. Error of Mean	0.1964	0.8609
Coefficient of variation	4.832%	24.03%

Experimental Details

- 40-µL dispense with 50-µL tips on ALH2
- 96-channel head
- Each data point is the average of 96 replicates from a different tip lot

Data Summary

- Current tips with this ALH may fall within specifications
- New tips do not meet specifications for both accuracy and precision.
- This ALH is clearly not performing optimally, but is much more sensitive to the tip source

Disposable Pipette Tip Verification Programs

Manufacturers

- Implement more robust QC program for manufacture of pipette tips
- **Benefit –** lot-based performance certification; Differentiate from other pipette tip manufacturers

Users

- Implement disposable pipette tip verification program allowing users to evaluate different manufacturing lots and compare between multiple sources.
- **Benefit** – ensure high quality data for long-term or continuous campaigns; decrease consumables cost by using universal tips or justify current consumables budget by using ALH vendor recommended tips.

Wrapping It Up…

1

MVS technology allows a facile method to qualify tips

2

Setting up a pipette tip verification program can be straightforward with the right tools – standardization is key

3

The quality of tips will affect your assay results – there are many factors to consider (not just cost or availability)

Thank you

Please contact my colleagues at Analis:

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