Quantifying the Impact of Pipette Tip Type Using Dual Dye Ratiometric Technology

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Abstract

The type, characteristics, and quality of disposable pipette tips can directly affect the volume transferred to and from an assay, regardless of operator skill or type of pipette employed. This application note will describe a way to correlate the accuracy and repeatability of volume transfers to the actual disposable pipette tips employed, which subsequently allows users to screen and select the appropriate tip types for their specific liquid handlers, pipettes and/or assays.

Introduction

All pipette tips are not created equal (see **Figure 1**), nor are all assays. Failure to use proper tips for a given type of pipette can lead to an inadequate seal between the pipette and tip, causing inaccurate volume transfers due to leakage and sample loss[1]. The need for the best performing tip is likely proportional to the importance of having *both* accuracy and precision in volume delivery for specific assay steps. For instance, dispensing relatively large volumes of phosphate buffer into a plate might not be as critical as dispensing 1 μL of compound for drug candidate screening or 15 μL of mastermix addition in PCR assays. Often, the driver for selecting, or switching, the type of tip is based on cost. Unfortunately, decisions to use new or different tip types are not usually based on tip performance, quality or reliability because these factors are not routinely characterized. This document gives examples of how testing volume transfer performance can give good indicators of tip performance and reliability. When tip reliability is in question, assay results, in turn, could be in question as well.

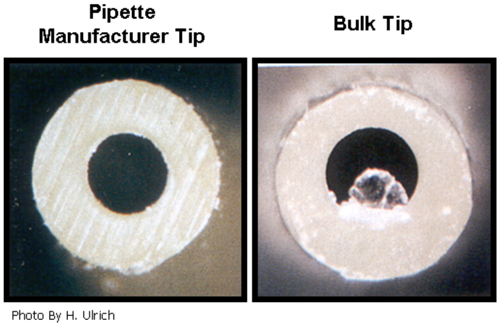


Figure 1. Cross-section of a properly manufactured disposable tip (left) and an improperly manufactured bulk tip (right). The manufacturer tip shows a centered orifice, has uniform appearance, is “finished” looking, and is often made from higher grade materials. The bulk tip shows a non-centered orifice with residual flashing material present. The bulk tip is also not as “finished” or “polished” looking. *Photos by Hans Ulrich*.

Artel’s dual-dye photometric technology for simultaneously measuring accuracy and precision of volume transfers allows users to objectively correlate volume transfer performance to tip type, enabling users to not only select the optimal tip type based on their needs, but also allows for troubleshooting specific volume transfer steps when tip reliability is in question.

Materials

- Artel PCS® Pipette Calibration System or MVS® Multichannel Verification System with appropriate microtiter plates
- Corresponding dye solutions for the target volume of interest for the PCS or MVS
- Pipette(s) and/or liquid handler(s)
- Appropriate disposable tip types for the pipette(s) and/or liquid handler(s)

Method

Experiment I.

The volumes dispensed by two different pipettes each using two different pipette tip types were compared. A standard pipetting method (described below) was used with each pipette to dispense 20 µL of Artel dye solution and the transferred volume was subsequently measured with the Artel PCS. *Pipette 1* was a 20-µL single-channel pipette dialed to 20 µL and *Pipette 2* was a 100-µL single-channel pipette dialed to 20 µL. For each pipette, one tip type was “manufacturer approved” for the specific pipette and the second tip type was generic. Both pipettes were from different manufacturers.

Important note: if the user intends to evaluate tip types with a handheld, manual pipette, the user should follow proper pipetting technique for pre-wetting, tip depth, pipette angle during aspiration, pause steps, etc. as discussed in reference 2. For all measurements, standard (forward) mode pipetting was employed:

- Load a new tip and properly pre-wet the tip by aspirating and dispensing the dye solution three times.
- With the plunger depressed to the first stop on the pipette, immerse the pipette tip under test 2 mm into the dye solution.
- During sample aspiration, the pipette should be vertical. Aspirate the solution by gently raising the plunger, keeping the tip immersed in the solution for 1 s before removing the pipette straight out of the liquid.
- Deliver the aliquot to the PCS vial by placing the pipette tip on the sidewall at a 45 degree angle just above the meniscus of the dye liquid in the vial. Slowly depress the plunger to the first stop and continue the plunger to the second stop on the pipette. Ensure that the entire aliquot is delivered to the vial.
- Remove the tip. Load a new tip and repeat.
- Collect 30 data points for each tip type on each pipette. Note – it is good practice to collect at least 6 to 10 data points per tip. When using high-density tip dispensers (i.e., 96 or 384-tips), it is common practice to balance time/costs by collecting three replicates per tip at a minimum.

Experiment II.

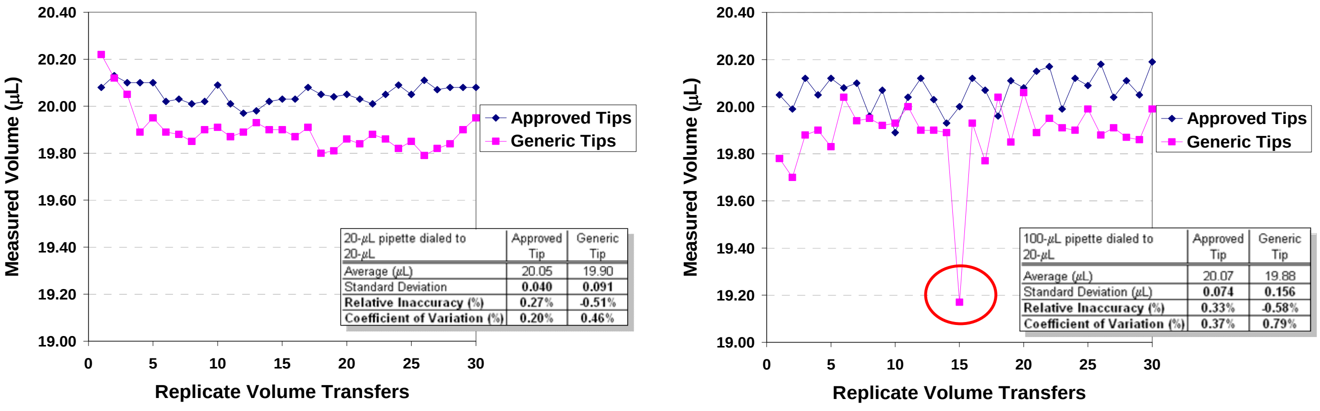
Three different tip types were compared using a 384-channel liquid handler. After a 384-tip head had gone through a preventive maintenance service from the manufacturer of the liquid handler, it was tested for dry dispensing 1 µL of DMSO dye solution into 384-well Corning 3711 flat-bottom microplates. In multiple liquid handler runs three different disposable tip types were employed: (a) manufacturer-approved 10-μL tips; (b) 10-μL tips from an alternative vendor X; and (c) 30-μL tips from an alternative vendor Y. Due to time constraints and amount of tips available, only two replicates were collected per tip, *i.e.*, two full 384-well plates were used to assess volume transfer performance based on tip type. The two destination plates, the tip types under test, and DMSO sample reservoir were placed on the deck of the liquid handler. The liquid handler was programmed to load tips, aspirate the DMSO solution, and dispense it into the first test plate. Before repeating the volume transfer to the second plate, the tips were discarded and new tips were loaded (i.e., non-prewetted, new tips were employed for single dispensing target volume for each replicate). For each testing experiment, *identical* liquid handler methods and parameters were employed. For instance, the aspirate rate(s), air gap volume(s), dispense rate(s), tip touch(es), etc. stayed exactly the same for every dispense and the only variable changed was the tip type. It is likely (but not known) that the head's Z direction (positioning up and down) could have been adjusted if the tips were of different lengths so that each tip type was almost pinned on the bottom of the well during the dispense. After two plates were filled for the first tip type, a second tip type was loaded onto the deck and the method was repeated (same for the third tip type). In all, six 384-well plates were filled. After the target volume of DMSO sample solution was dispensed, 54 μL of MVS Diluent (non-quantitative addition) was dispensed into every well of the 384-well plates using a Thermo Mutlidrop dispenser. The volumes were measured on a well-by-well basis using the MVS.

Experiment III.
An issue with tip reliabilty was identified when an 8-channel liquid handler was using tips manufactured by an alternative vendor. An 8-tip liquid handler was programmed to aspirate a target volume of Artel dye solution and dispense it into a microplate using generic disposable tips situated in 96-tip boxes. The tips were not from the manufacturer of the liquid handling equipment. The liquid handler transferred aliquots of dye solution (either 1 or 15 μL) into a Diluent-filled 96-well Artel Verification Plate, i.e., target volume was wet-dispensed into Diluent. Twelve replicates were dispensed per tip filling an entire plate. Each disposable tip was used once and discarded before transferring the next replicate sample. For each new test plate, a new box of tips was loaded onto the deck of the liquid handler. All test plates were measured with the MVS and all generic tips were from the same lot. Note – only generic tips were employed; this study did not compare different tip types.

Results

Experiment I.
The results for the manual pipetting[2,3] experiments indicate that in each example, the vendor approved pipette tips showed slightly better volume transfer peformance compared to generic tips for both the 20-µL pipette dialed to 20 µL (**Figure 2, left**) and the 100-µL pipette dialed to 20 µL (**Figure 2, right**). In both cases, the generic tips demonstrated a higher degree of variaiblity compared to the manfacturer approved tips (demonstrated by higher CVs, see inset tables in **Figure 2**) using the same pipetting technique[2,3] and conditions. Additonally, for one generic tip in the 100-µL pipette experiment, the volume transfer performance was noticably different than the rest (replicate 15, **Figure 2, right**).

Experiment II.
Three different tip types were evaluated and directly compared through experiments performed on a 384-channel automated liquid handler. The three tip types were each from a different source: (a) 10-µL manufacturer approved tips; (b) 10-µL tips from an alternative vendor X; and (c) 30-µL tips from an alternative vendor Y. The liquid handler was programmed to transfer 1 µL of DMSO dye soluton with the only difference between runs being the tip type loaded onto the deck of the liquid handler. Volume transfer performance values were directly compared for each tip type (**Table 1**). The user's goal was to evaluate tips from different sources for economic reasons with the hope of maintaining the same, or nearly the same, volume transfer performance. Based on the performance results – specifically CV values across the plate – the laboratory choose to use the 10-µL tips from alternative vendor X for the 1-µL DMSO transfer. In other collected data (not shown), the %CV values for vendor X's tips were not as good as CV values for manufacturer approved tips when dispensing < 1 µL (300 and 500 nL). In the end, this laboratory chose to use different tip types for different volumes

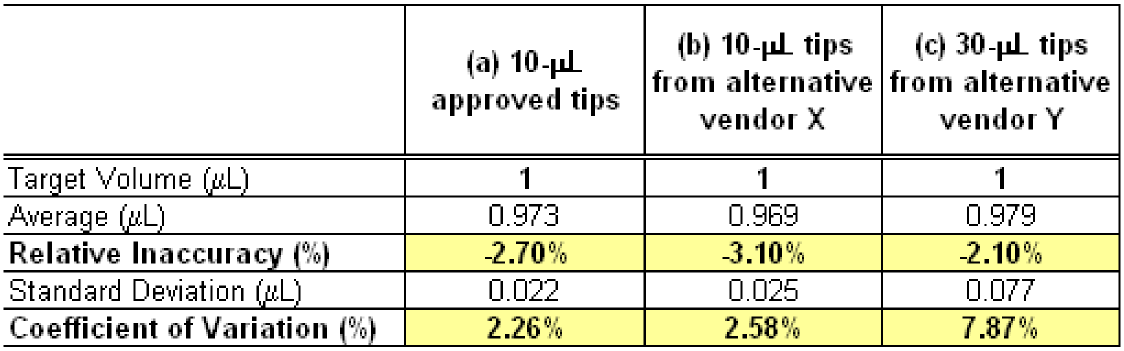


20-µL pipette dialed to 20-µL	Approved Tip	Generic Tip
Average (µL)	20.05	19.90
Standard Deviation	**0.040**	**0.091**
Relative Inaccuracy (%)	**0.27%**	**-0.51%**
Coefficient of Variation (%)	**0.20%**	**0.46%**

100-µL pipette dialed to 20-µL	Approved Tip	Generic Tip
Average (µL)	20.07	19.88
Standard Deviation (µL)	**0.074**	**0.156**
Relative Inaccuracy (%)	**0.33%**	**-0.58%**
Coefficient of Variation (%)	**0.37%**	**0.79%**

Figure 2. Comparing two disposable pipette tip types for two different handheld, single channel pipettes from diferent manufacturers. A 20-µL pipette dialed to 20 µL with both generic tips and manufacturer-approved (left). A 100-µL pipette was dialed to 20 µL, also with approved and generic tips (right).

Table 1. Evaluating Three Different Tip Types on a 384-tip robot in a high-throughput laboratory

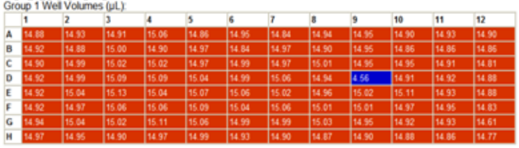


	(a) 10-µL approved tips	(b) 10-µL tips from alternative vendor X	(c) 30-µL tips from alternative vendor Y
Target Volume (µL)	1	1	1
Average (µL)	0.973	0.969	0.979
Relative Inaccuracy (%)	**-2.70%**	**-3.10%**	**-2.10%**
Standard Deviation (µL)	0.022	0.025	0.077
Coefficient of Variation (%)	**2.26%**	**2.58%**	**7.87%**

and therefore maintained a cost effective approach while remaining within their tolerances for volume transfer accuracy and precision for multiple target volumes.

Experiment III.
For the 8-tip automated liquid handler using generic tips, volume transfer results indicated that there was a *bad* tip within the lot. The tip issue was discovered through trial and error experiments when the 8-tip robot was used to dispense either 1 or 15 µL of sample into a 96-well plate. Upon inspection, one well continued to exhibit a lower measured volume relative to all other wells (well D9, see **Figure 3, Runs A** and **B**). In a follow-up experiment, it was observed that well E4 was demostrating the same behavior. In this situation it was determined that the tip box had been rotated 180 degrees on the deck before the volume transfer task had begun. Upon rotating the well-by-well results 180 degrees, well E4 directly correlates to well D9 (**Figure 3, Run C**). By having a rotated tip box on the deck, the actual robot channel (tip 4) was shown to not be the problem. In the last experiment, the tip from location D9 was literally swapped (by hand) with the tip in location D1 in the tip box before initiating the volume transfer task. After swapping tips in the tip box, the volume transfer results show that the outlier behavior migrated to well D1 (**Figure 3, Run D**). The testing and troubleshooting indicated that the disposable tip in location D9 was a “bad” tip. Upon inspection, it appeared as though this tip did not seat properly and the sample volume could not be fully aspirated (poor tip-to-channel seal). Had the troubleshooting experiments not been performed, the robot (and dispensing head) could have been incorrectly blamed for the erroneous results. *The actual disposable tip was to blame – not the liquid handler*[4,5]. The tips from this tip lot were not reliably manufactured. The effect of the bad tip on performance results is very noticeable when CVs are calculated with and without the low well (refer to tables per run in **Figure 3**). Note that Run C is for a 1-µL dispense while Runs A, B and D are for 15-µL dispenses making the difference in accuracy and precision values noticeable, i.e., the relative inaccuracy values for the 1-µL transfer are higher.

Run A



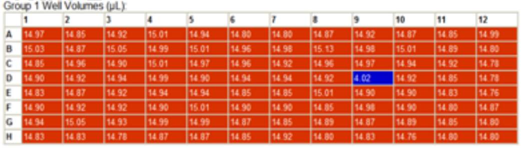
Group 1 Well Volumes (µL):

	1	2	3	4	5	6	7	8	9	10	11	12
A	14.88	14.93	14.91	15.06	14.86	14.95	14.84	14.94	14.95	14.90	14.93	14.90
B	14.92	14.88	15.00	14.90	14.97	14.84	14.97	14.90	14.95	14.86	14.86	14.86
C	14.90	14.99	15.02	15.02	14.97	14.99	14.97	15.01	14.95	14.95	14.91	14.81
D	14.92	14.99	15.09	15.09	15.04	14.99	15.06	14.94	4.56	14.91	14.92	14.88
E	14.92	15.04	15.13	15.04	15.07	15.06	15.02	14.96	15.02	15.11	14.93	14.88
F	14.92	14.97	15.06	15.06	15.09	15.04	15.06	15.01	15.01	14.97	14.95	14.83
G	14.94	15.04	15.02	15.11	15.06	14.99	14.99	15.03	14.95	14.92	14.93	14.61
H	14.97	14.95	14.90	14.97	14.99	14.93	14.90	14.87	14.90	14.88	14.86	14.77

Target volume = 15 µL	**All Data**	**Without Bad Tip Data**
Average (µL)	14.85	14.96
Standard Deviation (µL)	1.06	0.08
Relative Inaccuracy (%)	**-1.01%**	**-0.29%**
Coefficient of Variation (%)	**7.17%**	**0.55%**

Tip ID	**Tip-by-Tip CV**	**Tip-by-Tip CV without Bad Tip Data**
1-3, 5-8	< 0.87%	(same)
4	21.32%	0.51%

Run B



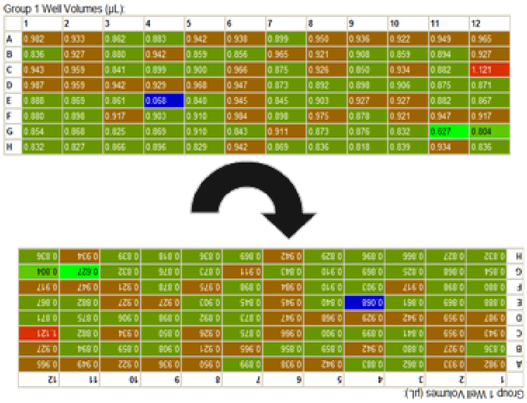
Group 1 Well Volumes (µL):

	1	2	3	4	5	6	7	8	9	10	11	12
A	14.97	14.85	14.92	15.01	14.94	14.80	14.80	14.87	14.92	14.87	14.85	14.99
B	15.03	14.87	15.05	14.99	15.01	14.96	14.98	15.13	14.98	15.01	14.89	14.80
C	14.85	14.96	14.90	15.01	14.97	14.96	14.92	14.96	14.97	14.94	14.92	14.78
D	14.90	14.92	14.94	14.99	14.90	14.94	14.94	14.92	4.02	14.92	14.85	14.78
E	14.83	14.87	14.92	14.94	14.94	14.85	14.85	15.01	14.90	14.90	14.83	14.76
F	14.90	14.92	14.92	14.90	15.01	14.90	14.90	14.85	14.98	14.90	14.80	14.87
G	14.94	15.05	14.93	14.99	14.99	14.87	14.85	14.89	14.87	14.89	14.85	14.80
H	14.83	14.83	14.78	14.87	14.87	14.85	14.92	14.80	14.83	14.76	14.80	14.80

Target volume = 15 µL	**All Data**	**Without Bad Tip Data**
Average (µL)	14.79	14.90
Standard Deviation (µL)	1.11	0.07
Relative Inaccuracy (%)	**-1.39%**	**-0.64%**
Coefficient of Variation (%)	**7.53%**	**0.50%**

Tip ID	**Tip-by-Tip CV**	**Tip-by-Tip CV without Bad Tip Data**
1-3, 5-8	< 0.60%	(same)
4	22.45%	0.37%

Run C



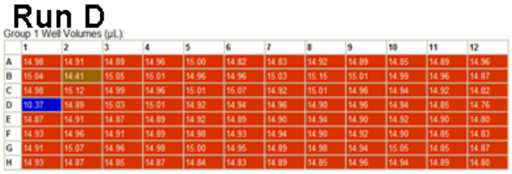
Group 1 Well Volumes (µL):

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.982	0.933	0.862	0.883	0.942	0.938	0.899	0.950	0.936	0.922	0.949	0.965
B	0.836	0.927	0.880	0.942	0.859	0.856	0.965	0.921	0.908	0.859	0.894	0.927
C	0.943	0.959	0.841	0.899	0.900	0.966	0.875	0.926	0.850	0.934	0.882	1.121
D	0.987	0.959	0.942	0.929	0.968	0.947	0.873	0.892	0.898	0.906	0.875	0.871
E	0.888	0.869	0.861	0.068	0.840	0.945	0.845	0.903	0.927	0.927	0.882	0.867
F	0.880	0.898	0.917	0.903	0.910	0.984	0.898	0.975	0.878	0.921	0.947	0.917
G	0.854	0.868	0.825	0.869	0.910	0.843	0.911	0.873	0.876	0.832	0.627	0.804
H	0.832	0.827	0.866	0.896	0.829	0.942	0.869	0.836	0.818	0.839	0.934	0.836

Target volume = 1 µL	**All Data**	**Without Bad Tip Data**
Average (µL)	0.89	0.90
Standard Deviation (µL)	0.10	0.06
Relative Inaccuracy (%)	**-11.10%**	**-10.24%**
Coefficient of Variation (%)	**11.45%**	**6.33%**

Tip ID	**Tip-by-Tip CV**	**Tip-by-Tip CV without Bad Tip Data**
1-3, 5-8	< 8%	(same)
4	29.16%	3.94%

Run D



Group 1 Well Volumes (µL):

	1	2	3	4	5	6	7	8	9	10	11	12
A	14.98	14.91	14.89	14.96	15.00	14.82	14.83	14.92	14.89	14.85	14.89	14.96
B	15.04	14.41	15.05	15.01	14.96	14.96	15.03	15.15	15.01	14.99	14.96	14.87
C	14.98	15.12	14.99	14.96	15.01	15.07	14.92	15.01	14.96	14.94	14.92	14.82
D	10.37	14.89	15.03	15.01	14.92	14.94	14.96	14.90	14.96	14.94	14.85	14.76
E	14.87	14.91	14.87	14.89	14.92	14.89	14.90	14.94	14.90	14.92	14.90	14.80
F	14.93	14.96	14.91	14.89	14.98	14.93	14.94	14.90	14.92	14.90	14.85	14.83
G	14.91	15.07	14.96	14.98	15.00	14.95	14.89	14.98	14.94	15.05	14.85	14.87
H	14.93	14.87	14.85	14.87	14.84	14.83	14.89	14.85	14.96	14.94	14.89	14.80

Target volume = 15 µL	**All Data**	**Without Bad Tip Data**
Average (µL)	14.88	14.92
Standard Deviation (µL)	0.47	0.09
Relative Inaccuracy (%)	**-0.82%**	**-0.50%**
Coefficient of Variation (%)	**3.18%**	**0.59%**

Tip ID	**Tip-by-Tip CV**	**Tip-by-Tip CV without Bad Tip Data**
1-3, 5-8	< 1.20%	(same)
4	9.05%	0.50%

Figure 3. Finding a "bad-tip-in-the-box" for generic liquid handler tips (not manufacturer tips for this robot). The overall plate results are shown with heat maps to help visually indicate where measured well volumes are different (i.e., in relative values – blue is low). In both replicate **Runs A** and **B**, well D9 was shown to be low relative to all other wells. In **Run C**, the tip box was rotated 180 degrees on the liquid handler deck; well E4 correlates to well D9 when the results are rotated 180 degrees. In **Run D**, the tip from location D9 was manually swapped with tip location D1 before performing the task.

Discussion and Conclusion

The data collected in the series of experiments illustrate an overall difference in the average delivered volume from a pipette or liquid handler when using disposable tips from different vendors. These results indicate the need for the user to verify pipette or liquid handler performance with the type of pipette tip employed. Volume transfer performance has been found to be directly related to tip quality because tip material, shape, properties, fit and wet-ability are all important factors for repeatability[4]. Even with the *best* liquid handler or pipette, different tip types will alter the volume transfer performance of the *system*. In a similar but different example, if someone wants to drive the most powerful automobile in an environment not suited for the tire type or tire quality, *i.e.*, racing tires used on snow-covered roads, the user will not get the best performance out of *that* system – unreliable tires will lead to non-optimal driving performance.

In all cases, the use of the dual-dye ratiometric photometry allowed the users to evaluate and identify appropriate tip types for their specific methods and target volumes of interest. With the magnitude of error and variability in the volume transfer performance solely due to the pipette tip, it is clear that there would have been negative impact on assay performance (or results interpretation) if the wrong tip was employed. Ultimately, without the testing that was performed, the liquid handler or pipette (or operator) could have been incorrectly blamed for poor performance when tip quality was to actually blame.

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