200 - 900 Nanoliter Differential Pipetting study quantitates the effects of aspiration and dispensing methodology on precision and accuracy and reflects the accuracy advantage of contact-free dispensing. Study performed May 20, 2015 in the Artel, Inc. Calibration Laboratory

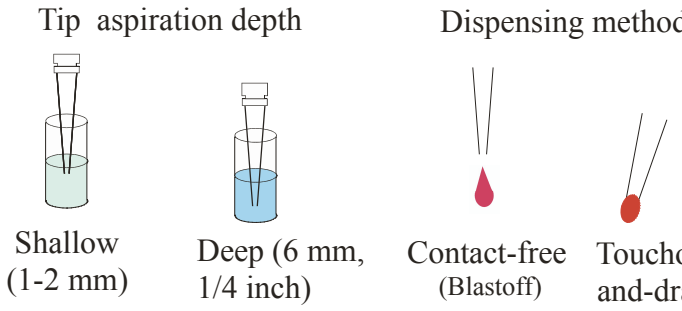
Donald Schwartz, M.D., President and Scientific Director, Differential Pipetting, Inc.
Travis Schafer, Quality Control and Field Applications Scientist, Artel, inc.

BACKGROUND, by Donald Schwartz. Differential Pipetting was invented and developed to provide a robust, practical and contamination-free way to precisely and accurately pipette the ever-smaller volumes desired by important new areas of medicine and science, such as NGS (Next Generation Sequencing) and HLA (Human Lymphocyte Antigen (aka tissue typing). We use weighing and the Artel PCS dual beam ratiometric system to document our precision and accuracy (**Reference 1**). In addition to correlations with conventional pipettors, we have recently published data at the 1µL level that specifically quantifies the additional volume that is picked up by dipping unduly deep during aspiration (**Reference 2**) and effects of the traditional touchoff dispensing process itself on the volume transferred (**Reference 3**). As Differential Pipettors are now in use at the 500 nanoliter level in the NGS and HLA area, I wanted to see to what extent we might be able to quantitatively define the combined matrix effects of aspiration dipping depth and touchoff vs contact-free delivery within the tight 200 to 900 nanoliter range.

I felt this demanding study could best be done by Travis Schafer in his tightly environmentally controlled Calibration Laboratory, and I gave him our first "ultra ultra" 0.2 - 1.6 µL Differential Pipettor to use for it. This unit has the incredible resolution that a ten µL (10µL!) syringe would have -- that is, 6 full mm of excursion/µL -- combined with the vigorous clean sample blowout ("Blastoff") that a 1 mL syringe or large volume pipettor could give. Though designed to dispense cleanly contact-free, the Differential Pipettor can also dispense by conventional touchoff-and-drag, thus eliminating any differences in aspiration and isolating any differences in transfer volume to the dispensing portion. And the Artel PCS dual beam ratiometric system, though much of its vial design is to thoroughly capture specimens touched off onto its walls, can also receive specimens directly into the vial liquid that are blown off through the air into it without any tip contact. So this was set up to get some very precise data. Measurements were made during a 1.5 hour morning session.

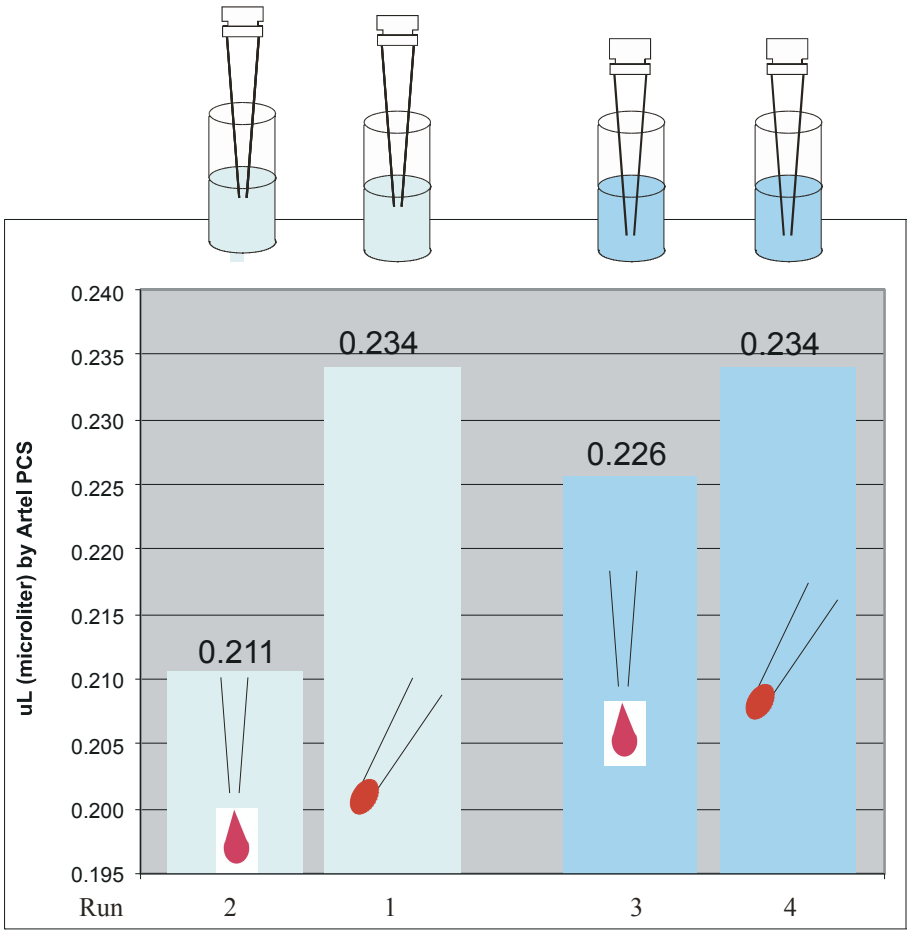
MATERIALS and METHODS, by Travis Schafer**.** The laboratory conditions and my standard pipetting technique were the same as previously described for a prior correlation study (**Reference 4**). Artel PCS used for measurements. Temperature 21.2 - 22.0 degrees C and 50-55% humidity. A new pure polypropylene LS3 Little Squirt tip was used each time (straight from the rack without any priming or other preparation). During aspiration, I held the pipettor tip reasonably vertically and immersed the tip 1-2 mm beneath the surface of the sample (dye), as I normally do for tiny samples. For dispensing by touchoff-and-drag, I used my usual technique of holding the tip at the minimal angle necessary to touch it off, which is as close to vertical as possible in the 10 - 20 degree range to minimize any tip flow restriction and optimize liquid transfer. For dispensing contact-free I did this from a comfortable but non-precision distance in an approximately vertical orientation.

At each volume level I did 4 series of 5-10 measurements each: dipping shallow with contact-free blowout, shallow with touchoff delivery, deep dip with contact-free blowout and then deep dipping with touchoff delivery.



Starting near the lowest 0.2 µL end we completed 4 combinations of 30 measurements in 13 minutes.

Run #	1	2	3	4
Time	8:51 AM	8:56 AM	8:59 AM	9:04 AM
Aspiration tip immersion	shallow 1-2 mm	shallow 1-2 mm	deep 1/4" (6 mm)	deep 1/4" (6 mm)
Delivery technique	Touchoff and drag	Contact-free Blast off	Contact-free Blast off	Touchoff and drag
Mean µL	**0.234**	**0.211**	**0.226**	**0.234**
Standard Deviation	0.0164	0.0150	0.0153	0.0184
CV% precision	7.0%	7.1%	6.8%	7.9%
# Points >2SD out rejected	0	1	1	1
# points used in calculation	5	7	9	9

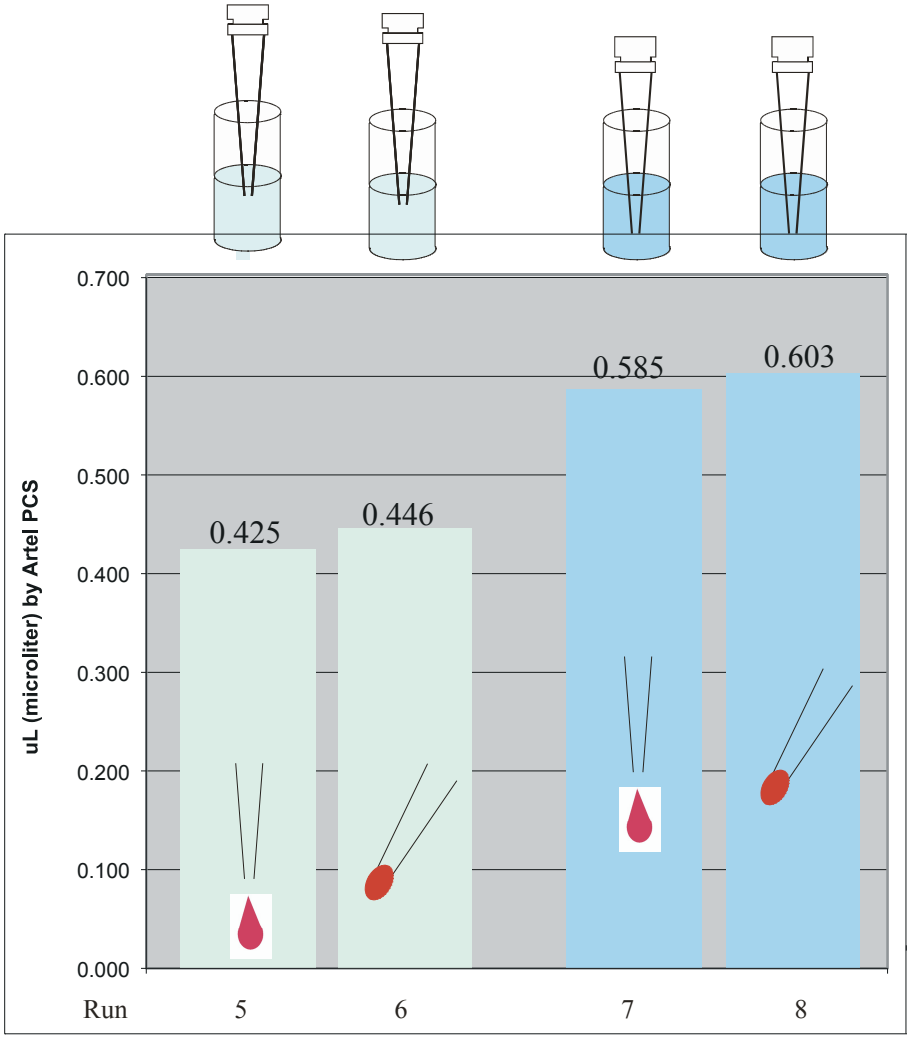


Change from contact-free			
absolute µL	0.023 µL		0.008 µL
relative %	11.1%		3.7%

Touchoff volumes are greater than the Differential Pipetting Contact-free "Blastoff" volumes -- 11% higher with shallow aspiration dipping and 4% higher with deep dipping. With conventional Touch off-and-drag dispensing some liquid on the *outside* of the tip gets drawn or "wicked" off onto the receiving surface. In Contact-free dispensing only the liquid that was aspirated *inside* the tip is dispensed -- cleanly and fully -- leaving behind the liquid that of necessity clung to the *outside* of the tip during aspiration. The dispensing difference is independent of the effect of gravity and hydrostatics, which causes additional liquid to be aspirated when dipping unduly deep during aspiration, regardless of how it is later delivered.

Up one level, completing 4 combinations of 39 measurements in the 0.4 µL range in 19 minutes.

Run #	5	6	7	8
Time	9:38 AM	9:46 AM	9:52 AM	9:57 AM
Aspiration tip immersion	shallow 1-2 mm	shallow 1-2 mm	deep 1/4" (6 mm)	deep 1/4" (6 mm)
Delivery technique	Contact-free Blastoff	Touchoff and drag	Contact-free Blast off	Touchoff and drag
Mean µL	**0.425**	**0.446**	**0.585**	**0.603**
Standard Deviation	0.0138	0.0242	0.0175	0.0255
CV% precision	3.3%	5.4%	3.0%	4.2%
# Points >2SD out rejected	1	0	0	0
# points used in calculation	9	10	10	10

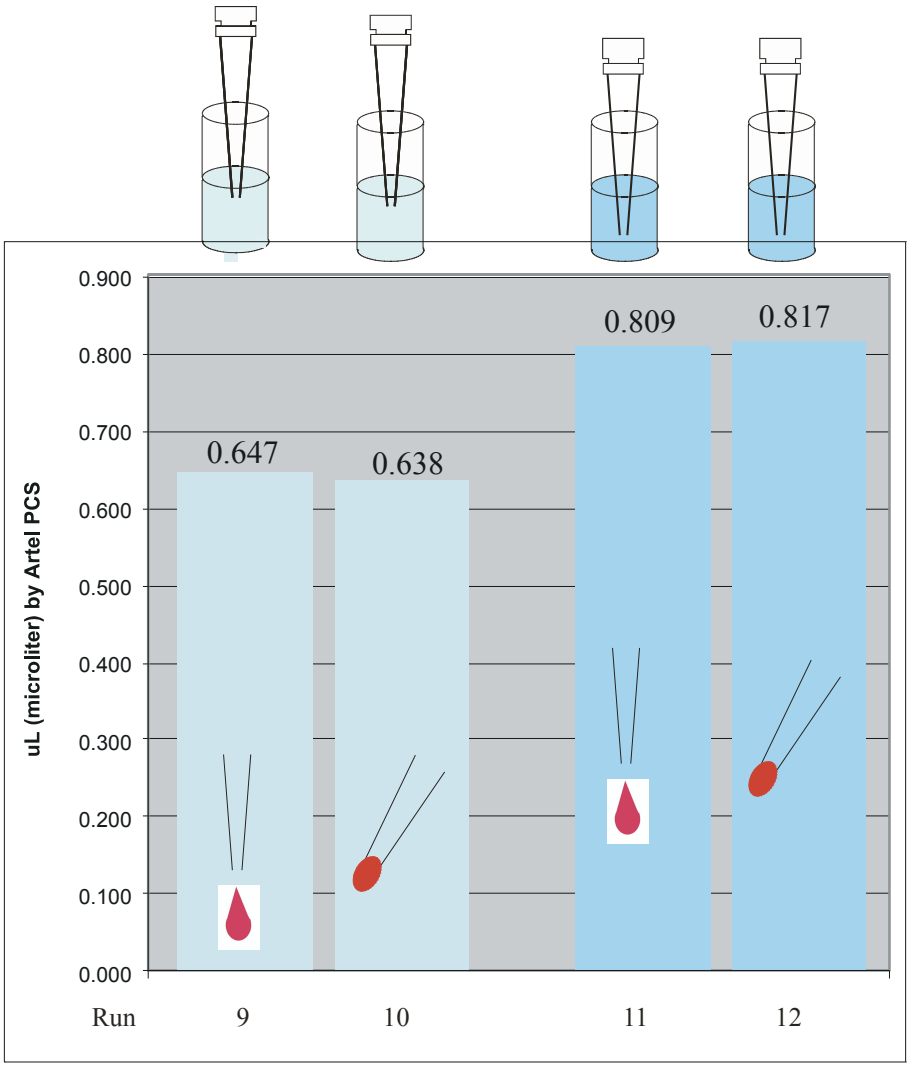


Touchoff volumes are greater than the Differential Pipetting Contact-free "Blastoff" volumes....5% higher for the shallow tip dip and 3% higher for the deep tip dip....consistent with material on the outside of the tip being wicked off in the touchoff runs but left behind in the contact-free Blastoff runs.

Change from contact-free		
absolute µL	0.021 µL	0.018 µL
relative %	4.8 %	3.1 %

Up one level, completing 4 combinations of 23 measurements in the 0.6 µL range in 9 minutes.

Run #	9	10	11	12
Time	10:03 AM	10:06 AM	10:09 AM	10:12 AM
Aspiration tip immersion	shallow 1-2 mm	shallow 1-2 mm	deep 1/4" (6 mm)	deep 1/4" (6 mm)
Delivery technique	Contact-free Blastoff	Touchoff and drag	Contact-free Blast off	Touchoff and drag
Mean µL	**0.647**	**0.638**	**0.809**	**0.817**
Standard Deviation	0.0082	0.0083	0.0157	0.0324
CV% precision	1.3%	1.3%	1.9%	4.0%
# Points >2SD out rejected	0	0	0	0
# points used in calculation	8	5	5	5



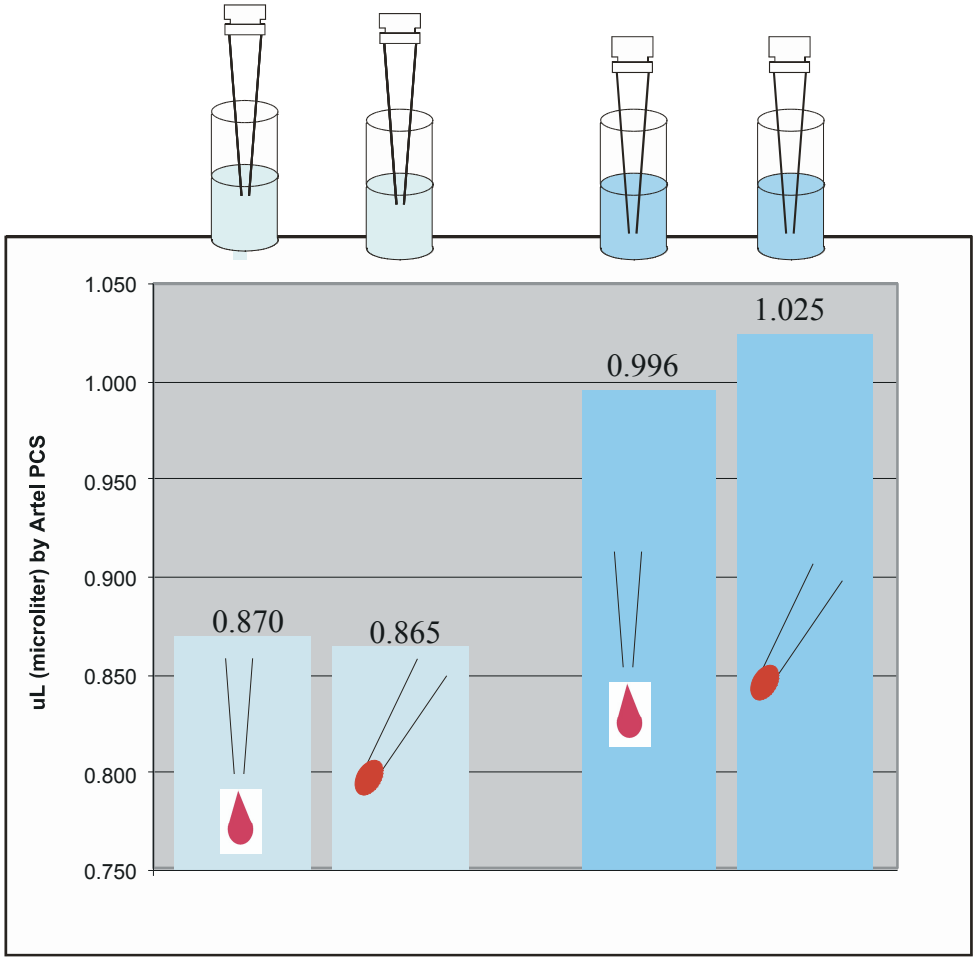
RESULTS and ANALYSIS.

For shallow tip dip, touchoff Run 10 is now 1.5% *lower* than the contact-free Run 9, but for the deep dips the previous pattern holds in which the touchoff is higher (by only 1%). This is probably because with the larger volume more liquid is being retained INSIDE the tip during touchoff than is being wicked off, but with the deep dip there is enough extra outside the tip to wickoff overpower (barely) the inside retention.

Change from contact-free			
absolute µL	-0.010 µL		0.008 µL
relative %	-1.5 %		1.0 %

Up one level, completing 4 combinations of 20 measurements in the 0.8 µL range in 7 minutes.

Run #	13	14	15	16
Time	10:18 AM	10:21 AM	10:23 AM	10:25 AM
Aspiration tip immersion	shallow 1-2 mm	shallow 1-2 mm	deep 1/4" (6 mm)	deep 1/4" (6 mm)
Delivery technique	Contact-free Blastoff	Touchoff and drag	Contact-free Blast off	Touchoff and drag
Mean µL	**0.870**	**0.865**	**0.996**	**1.025**
Standard Deviation	0.0144	0.0157	0.0179	0.0084
CV% precision	1.7%	1.8%	1.8%	0.8%
# Points >2SD out rejected	0	0	0	0
# points used in calculation	5	5	5	5

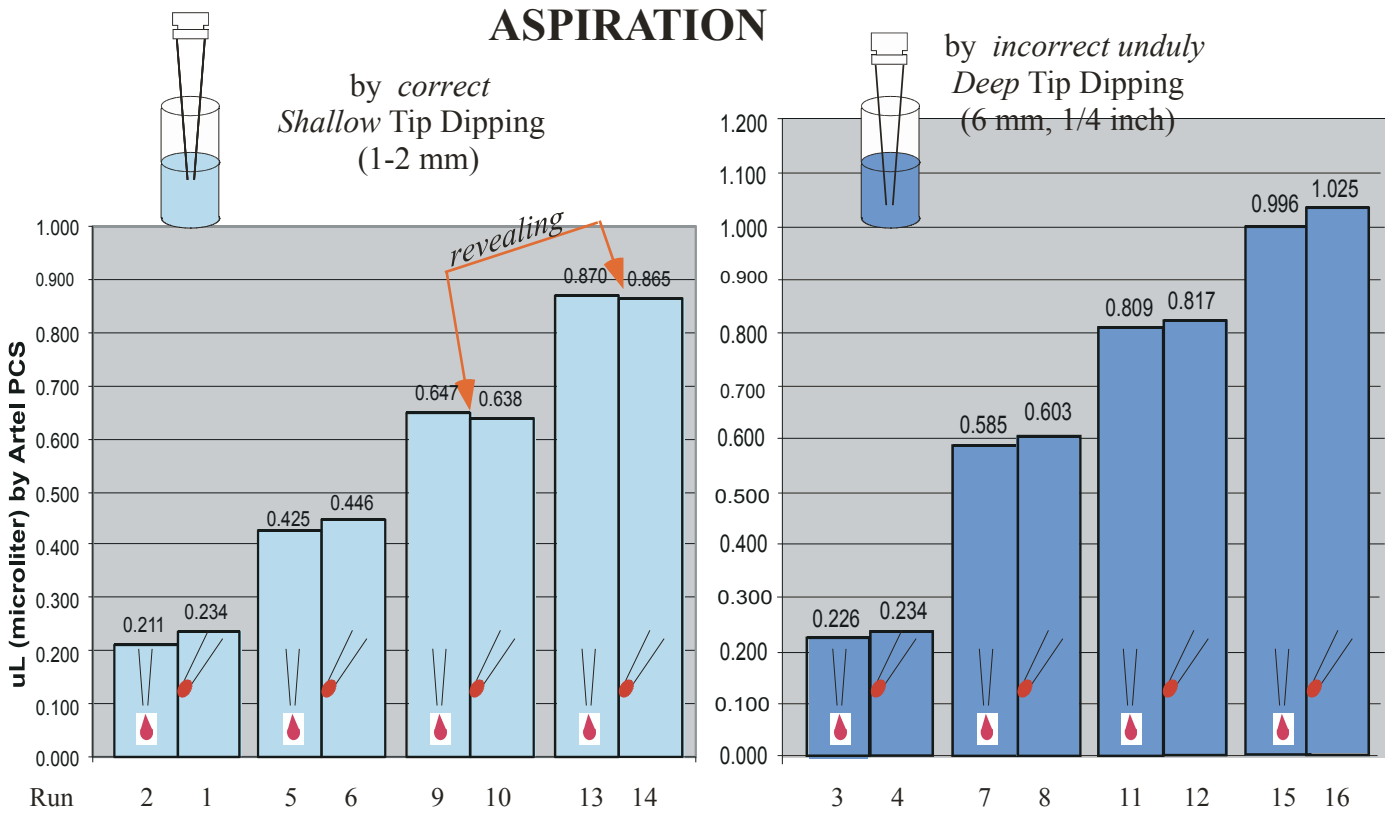


Change from contact-free		
absolute µL	-0.005 µL	0.029 µL
relative %	-0.6 %	2.9 %

RESULTS and ANALYSIS.

For the shallow aspiration dip, the pattern of the 0.6 uL range is repeated in that the touchoff is 0.6% lower than the contact-free blowoff. For the variable deeper dip, the touchoff is 3% higher than the contact-free blowoff, same pattern as for 0.6uL range and same logical explanation -- that the much larger volume inside permits more retention inside than is wicked off outside, but with the deep dip there is enough extra outside the tip to wickoff overpower the inside retention.

OBSERVATIONS and RESULTS . All the data was collected in 1.5 hours and is summarized in the bar graphs below to show the shallow and deep aspiration tip-dipping runs separately. Following deep aspiration dipping, conventional touchoff-and-drag dispensing transferred ***more*** volume than the contact-free dispense at all volumes. Following shallow aspiration dipping, the two highest volumes delivered ***less*** volume than the contact-free dispense, a reverse pattern.



DISPENSING for each volume level is shown by Contact-free "Blastoff" followed by conventional Touchoff-&-Drag.

Touch off-and-drag dipensing as done by conventional pipetting and as can also be done by differential pipetting.

Contact-free ("Blastoff") pipetting dispensing by Differential Pipettor.

DISCUSSION. Ideally, aspiration picks up just the amount one wants and delivers just that amount. This is what we believe the Differential Pipettor's crisp contact-free dispense enables-- cleanly picking up "HERE" and delivering all of what was aspirated inside "THERE" -- leaving behind any liquid clinging to the outside of the tip. Traditional touchoff-and-drag dispense is expected to ADD some additional volume during dispensing from liquid clinging to the outside surface of the tip that is "wicked off" when it contacts the receiving surface. Sometimes some liquid is retained inside the tip, particularly when the delivery speed is sluggish, thus REDUCING the intended volume transfer. Outside "wickoff" and inside "retention" can therefore operate in opposite directions and modify or corrupt the intended pipetted volume. With good technique, the "wickoff" volume is hopefully very small, but it is well known enough that some pipetting procedures instruct to quickly wipe down the outside of the tip to remove such clinging liquid before delivering it so that it cannot be dragged off. Likewise, it is also appreciated that sluggish delivery -- common with small volume pipettors with their obligatory very small cross section pistons -- can encourage inside retention.

The reverse behavior that the precision of this study shows in runs 10 and 14 is thought-provoking and probably just reflects a reversal of the balance between outside "wickoff" addition and inside retention reduction. When the tip dip remains shallow but much more is aspirated in runs 10 and 14 then there is still just a tiny amount on the outside to "wickoff" but a lot more inside for more to be retained -- and the balance comes out a reduction. But when the tip dip is deep as it runs 12 and 16 there is logically enough more liquid clinging outside to enable the tip wickoff on delivery to overpower the inside retention. The balance between internal retention and external wickoff of liquid may well be governed by various factors that tip it one way or the other other, such as humidity, barometric pressure, temperature, liquid viscosity, etc.

It is obviously beyond the scope of this study to do more than speculate on which way the outside "wickoff"/inside "retention" balance will tip and under what conditions. But we do know that with the contact-free dispensing in the Differential Pipettor, the very first impetus the liquid in the tip receives to move down is a strong and crisp smack, which sends all of the aspirated contents South before there is much chance for any liquid to cling to the inside. We also know that inspection of the tip following the blowout occasionally shows some of the strong red dye remaining on the outside of the tip but almost never inside. This is in contrast to standard pipettor touchoff-and-drag dispensing, in which the liquid in the tip can only be moved down slowly (because only a single and relatively fine resolution mode is available) letting some layers cling closely to the inside of the tip even as there is "wickoff" from the outside -- a potentially fussy balancing act.

CONCLUSIONS. This is probably the first time that the effects of aspiration dipping depth and contact-free vs touchoff-and-drag dispensing have been studied quantitatively in anything approaching this small volume range. We believe that the contact-free dispensing method used with the Differential Pipettor gives truer and more accurate results and is free from the variation in dispensing technique from different operators that inevitably accompany traditional touchoff-and-drag dispensing.

REFERENCES.

Reference 1 Use of Artel PCS for receiving dispensed samples contact-free vs from touchoff-and-drag
www.Differential Pipetting.com SCIENCE 2

Reference 2 Improving accuracy of small volume pipetting by not dipping too deep during aspiration
www.Differential Pipetting.com SCIENCE 1

Reference 3 Are you really pipetting 1 µL? Combined effects of aspiration depth and dispensing method
www.Differential Pipetting.com SCIENCE 4

Reference 4 0.75µL-10µL correlation between Differential Pipettor and Gilson and Rainin pipettors
www.Differential Pipetting.com SCIENCE 7