Improving accuracy of small volume pipetting by not dipping too deep. Differential Pipettor study with contact-free dispensing at just below 1 µL shows that dipping the tip unduly deep during aspiration increased the volume that was aspirated due to gravity/hydrostatic effects.

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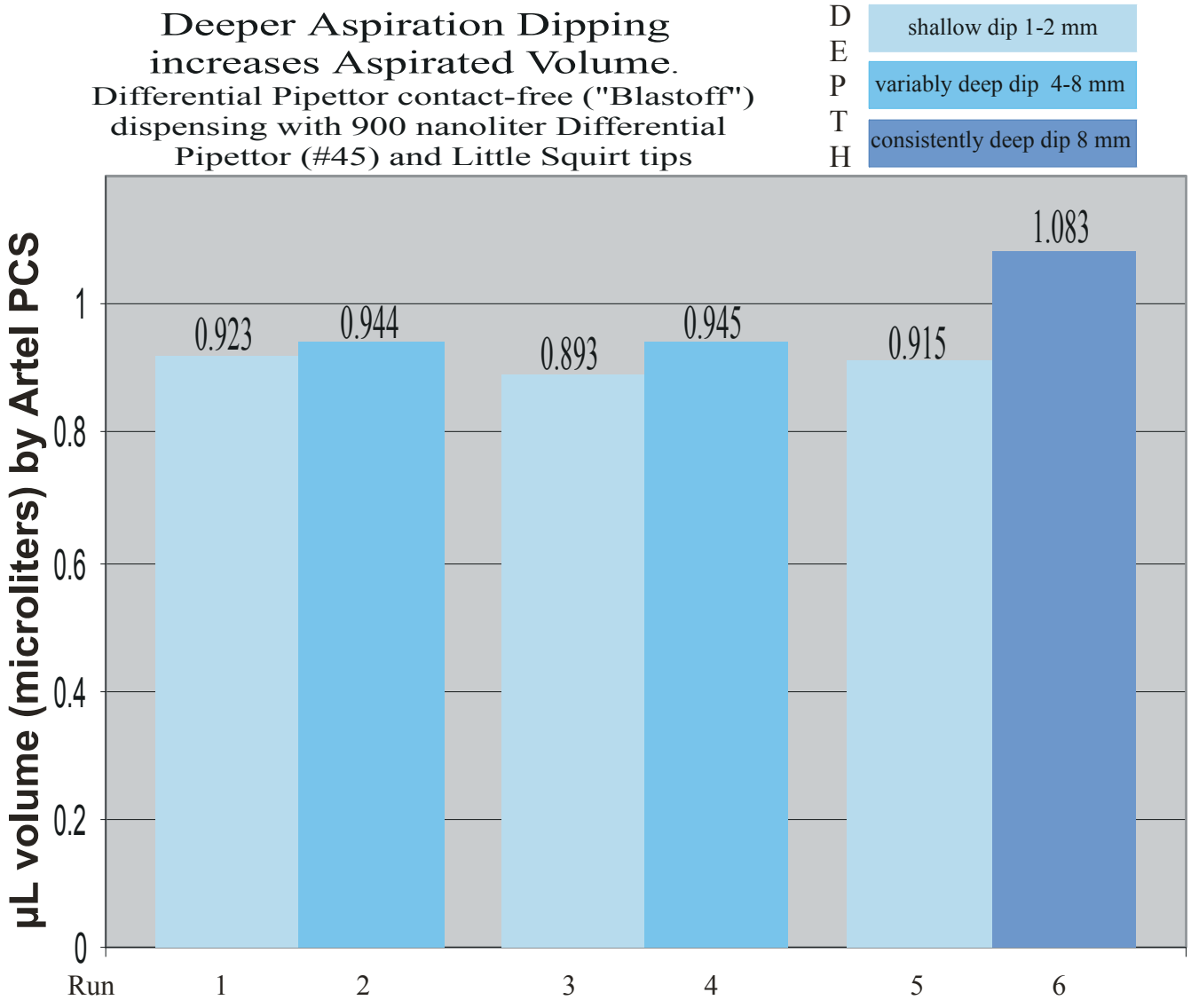
We have used the Artel PCS for low-volume studies (Reference 1). A recent pipetting comparison study against the Differential Pipettor suggested that how deep the tip is dipped during aspiration affected pipetting results for small volumes. Specific guidelines for dipping depth are abundant from Artel (Reference 2), Gilson (Reference 3), Eppendorf (Reference 4), Rainin (Reference 5) and others. However, the data underlying these guidelines comes from touchoff and immersion dispensing, which introduces other factors. We decided to exploit our ability to dispense contact-free, without external liquid wickoff or related effects, to try to get at the depth effect directly. We used a DRP 0.9 µL (Differential Pipettor #45 calibrated for 900 nanoliters) and our Little Squirt tips. During aspiration we dipped the tip about 1-2 mm, holding the pipettor and tip substantially vertically, as should be done for this small volume. Then we dipped *variably* deeper in the 4-8 mm range as we thought people might do. Then we dipped 8 mm *consistently*. Dispensing was done in our usual contact-free "Blastoff" mode, blowing the sample cleanly off the tip directly into the Artel PCS vial liquid without the tip touching anything. Volume used for each run is the mean of 8-12 measurements.



Date of study	4/17/15	4/17/15	4/20/15	4/20/15	4/19/15	4/19/15
Run #	1	2	3	4	5	6
Tip dip during aspiration	1-2 mm	4-8 mm	1-2 mm	4-8 mm	1-2 mm	8 mm
Dispensing method	Blastoff	Blastoff	Blastoff	Blastoff	Blastoff	Blastoff
Target volume µL	0.900	0.900	0.900	0.900	0.900	0.900
Tip depth dipping	consistently	variably	consistently	variably	consistently	consistently
Mean	**0.923**	**0.944**	**0.893**	**0.945**	**0.915**	**1.083**
Relative inaccuracy %	2.6%	4.9%	-0.8%	5.0%	1.6%	20.3%
CV% precision	**1.5%**	**3.8%**	**2.1%**	**4.4%**	**2.3%**	**2.6%**
Temp deg C	24.3	23.6	23.0	23.0	23.2	23.3
Total points run	12	10	10	9	9	9
# Outliers rejected for >2SD	0	0	1	1	0	0
# points used in calculation	12	10	9	8	9	9
µL increase from deeper dip		0.021		0.053		0.168
% vol increase fr deeper dip		2.2%		5.6%		15.6%

RESULTS and CONCLUSIONS. Dipping the tip unduly deep in the liquid being aspirated increases the volume aspirated, a know gravity/hydrostatic effect. The two runs in which we intentionally let our deeper dips vary (runs 2 and 4) had the two highest CVs, reflecting the actual greater variability in the volumes aspirated. Conversely, the 3 runs (1,3 and 5) in which we dipped only the needed 1-2 mm had the lowest CVs .

Generally, at about the 1 µL level, lapsing a little casual or tired can easily vary aspiration depth dipping enough to add another 3% accuracy error, and a hands-on regulatory overseer could easily rack up a 15% error. Dipping 1-2 mm during aspiration while holding the Differential Pipettor reasonably vertically is actually quite practical and will give accuracy and precision like the above.

REFERENCES.

Reference 1 www.DifferentialPipetting.com Science section item SCIENCE 2.

Reference 2 www.Artel-USA.com 10 Tips, Tip 8 “immerse the tip to the proper depth”.

Reference 3 Gilson Pipetman “Guide to Pipetting” chapter 2 aspiration depth table for different volumes.

Reference 4 Eppendorf “Impact of pipetting techniques on precision and accuracy” table for optimal immersion depth for different pipetting volumes and some quantification.

Reference 5 Rainin Pipetting Seminar has aspiration depth tables for different volumes.

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