Differential Pipetting

1 µL and 20 µL Pipetting Precision and Accuracy Comparison between the Gilson Pipetman and the Differential Pipettor, at the Whitehead Institute Ploegh Lab.

by Donald Schwartz, M.D., President and Scientific Director 4/16/2015 R1/30/2016

BACKGROUND: On Jan 21, 2015, Whitehead Institute postdoctoral fellow Florian Schmidt had pipetted 20 µL with the Differential Pipettor (adjustable 2-20 µL model DP20) and his reference Gilson Pipetman P20, and the data came out that both pipettors gave substantially the same excellent precision and accuracy. Today's study repeats that but adds a 2nd operator, visiting fellow Merte Bos, with both operators repeating the measurements at 20 µL but also pipetting 1 µL. The 20 µL reference pipettor Flo selected is the same trusted Gilson Pipetman P20 with USA ScientificTipOne 1-200 uL filter tips that he used on Jan 21st. For the 1 µL pipetting, the reference pipettor he selected is the Gilson Pipetman P2 with USA Scientific TipOne 0.1-10/20 µL filter tips. For the Differential pipetting, a fixed volume DP20 unit was used for 20 µL and a fixed volume DP2 unit was used for 1 µL, both with LS-3 tips. I brought in an Artel PCS Pipette Calibration System, a ratiometric photometry method, to quantify the pipettings, and did a quick check on it before the main runs. Very brief instructions were given to Flo and Merte to dispense into the PCS vial with the Gilson units by their standard touchoff-to-the-side-and-drag method, and to dispense with the Differential Pipettors by pointing the tip towards the vial center and blowing off contact-free. No special aspiration technique instructions were given. The idea was for Flo and Merte to run on their own, including running the Artel PCS for their own measurements, and see how things came out. Ploegh Lab Manager Robert Miller and I observed the runs.

RESULTS

	Don quick PCS check before runs		By Flo				By Merte				By Don	
			20µL		1µL		20µL		1µL			
Run	1	2	3	4	5	6	7	8	9	10	11	12
Time	10:52 AM	10:56 AM	11:14 AM	11:21 AM	11:34 AM	11:41 AM	11:51 AM	12:03 PM	12:19 PM	12:24 PM	12:47 PM	12:53 PM
Operator	Don	Don	Flo	Flo	Flo	Flo	Merte	Merte	Merte	Merte	Don	Don
Pipettor	Dif Pip	Dif Pip	Gil P-20	Dif Pip	Gil P-2	Dif Pip	Gil P-20	Dif Pip	Gil P-2	Dif Pip	Gil P-2	Dif Pip
Tip	LS-3	LS-3	TipOne	LS-3	TipOn e	LS-3	TipOne	LS-3	TipOn e	LS-3	TipOne	LS-3
Target volume	20 µL	1 µL	20 µL	20 µL	1 µL	1 µL	20 µL	20 µL	1 µL	1 µL	1 µL	1 µL
Dispense type	Contact-free Blastoff	Contact-free Blastoff	Touchoff & drag	Contact-free Blastoff	Touchoff & drag	Contact-free Blastoff	Touchoff & drag	Contact-free Blastoff	Touchoff & drag	Contact-free Blastoff	Touchoff & drag	Contact-free Blastoff
												
Mean result	**19.97**	**0.991**	**19.96**	**19.86**	**0.987**	**1.03**	**19.89**	**19.89**	**0.989**	**0.986**	**0.994**	**0.955**
Relative innac	-0.17%	-0.90%	-0.21%	-0.68%	-1.30%	2.70%	-0.50%	-0.57%	-1.08%	-1.40%	-0.62%	-4.52%
CV%	**0.18%**	**1.75%**	**0.19%**	**0.31%**	**1.57%**	**3.80%**	**0.25%**	**0.16%**	**1.04%**	**3.47%**	**2.03%**	**1.31%**
Temperature	21	21.2	21.8	22.1	22.5	22.7	22.5	23	23.4	23.5	23.9	23.9
Total points	3	6	13	13	11	10	13	14	10	11	10	11
>2SD outliers	0	0	0	0	0	0	1	0	0	1	0	0
Points used	3	6	13	13	11	10	12	14	10	10	10	11

OBSERVATIONS and an extra run. Precision looked great right from the first few pieces of data that came out from Flo's initial run. Flo and Merte blitzed through their runs, with the PCS feeding out impressive looking statistical results, and left to continue their regular work. When I then looked the data over I told Robert Miller that I didn't know why the Differential Pipettor's 1 µL CVs were higher than our 2% spec and commented that the aspirations had been done at a steep angle. Robert may have also noted that. He said "***does it matter?***" After a few seconds I answered "***it shouldn't***", but I was bothered enough to delay packing up to squeeze in one pair of 1 µL runs myself in which I held the pipettors and the tips approximately vertically and dipped shallow about 1-2 mm when aspirating, per industry recommendations for small volumes and what I am accustomed to doing.

ANALYSIS

The precision and accuracy data overall is really terrific for all 4 pipettors used. The 20 µL precision champion was Merte's Differential Pipettor 0.16% CV in run 8. The 1 µL precision champion was Merte's Pipetman 1.04% CV in run 9. So Merte was the human champ.

But it stands out that Flo and Merte's 1 µL CVs with the Differential Pipettor (runs 6 and 10) were both about 3.5%, which is outside our 2% spec. But I got 1.3% in the final run 12, and even my initial PCS rough checkout run 2 (before the PCS had really settled in) had a 1.8% CV, and in both of these I aspirated straight and to about 1-2 mm depth. Why the difference?

I quickly realized that my "***it shouldn't***" answer to Robert Miller's question was wrong and that steep aspiration angle and variable depth likely associated with it matters a lot. A major Artel study (**Reference 1**) got 1 µL CVs of 1.6% and 1.2% with our adjustable 10 µL and 5 µL Differential Pipettors and all aspirations in that study were with the tips approximately vertical and to the 1-2 mm depth. Industry literature generally notes the importance of aspirating straight and shallow (**References 2,3 and 4**) but appears light on actual data, understandably difficult to come by at the 1 µL level without our technology. During the next 3 days we therefore did a study (**Reference 5**) elucidating the deep-dip effect which also showed that aspiration dipping variably in the 4-8 mm range (runs 2 and 4) caused 1 µL CVs to jump to 4%, probably explaining the Whitehead 3.5% data. We further elucidated this in a study that combined the effects of aspirating deep and variably with errors from touchoff-and-drag dispensing itself (**Reference 6**). References 5 and 6 may be groundbreaking studies.

The moral of all this? If I -- who am steeped in this -- could forget and erroneously think "***it shouldn't***" for even 5 minutes then exceptional Whitehead fellows and excellent pipettors could easily make the same mistake. Could it be that 95% of good pipetting people worldwide make the same mistake every day? Could this be because *conventional pipettors force people to dispense at an angle* and the powerful habit carries over to aspiration, overpowering consistent manufacturer instructions to aspirate vertically/straight? If so, one might say that Differential Pipetting is more natural in facilitating a consistent and therefore habit-reinforcing straight- is-best practice for both aspiration and dispensing.

Then again, maybe the ingrained habit of aspirating at an angle comes from its being easier to see the tip to make sure that aspiration didn't fail; this happens with tiny volumes from seal leak with conventional pipettors, leading to duplicate and triplicate practices, but is extremely rare with differential pipetting. At the 20 µL level any error is obviously tiny and this doesn't seem to matter. But at 1-2 µL and smaller the aspiration angle and depth significantly affect results and it behooves one to remember "Yes, Virginia, of course accuracy matters" down in that world (**Reference 7**).

REFERENCES.

Reference 1 Artel study 1/23/2015
www.DifferentialPipetting.com SCIENCE 7

Reference 2 www.Artel-usa.com "10 Pipetting Tips" emphasizes aspirating straight even though conventional pipetting requires dispensing on an angle.

Reference 3 Gilson "Guide to Pipetting" Chapter 2 notes importance of tip immersion depth and handling angle.

Reference 4 Raining Pipetting Seminar, slide 11 "tip immersion angle is one of the most common incorrect pipetting methods".

Reference 5 "Improving Accuracy of Small Volume Pipetting by Not Dipping Too Deep", www.DifferentialPipetting.com SCIENCE 1

Reference 6 "Are You Really Pipetting 1 µL?" www.DifferentialPipetting.com SCIENCE 4

Reference 7 "Yes, Virginia, of course accuracy matters". www.DifferentialPipetting.com SCIENCE 3

ACKNOWLEDGMENTS and CREDITS: I thank Hidde Ploegh, Director of the Ploegh Lab at the Whitehead Institute, for graciously affording us this opportunity. I thank Lab Manager Robert Miller for his good question, and Florian Schmidt and Merte Bos. I particularly thank admin Beverly Dobson for her aplomb and chocolate cake. PCS® is a registered trademark of Artel, Inc. 25 Bradley Drive, Westbrook, ME 04092 www.artel-usa.com. Differential Pipettor™ , Differential Pipetting™ Blastoff™ and Little Squirt™ are trademarks of Differential Pipetting, Inc. Pipetman® is a registered trademark of Gilson S.A.S. TipONE® is a registered trademark of USA Scientific.

Differential Pipetting, Inc 11 Dory Road, Blackburn Industrial Park, Gloucester, Mass 01930 USA
www.DifferentialPipetting.com Science@DifferentialPipetting.com **(978) 515-3392**