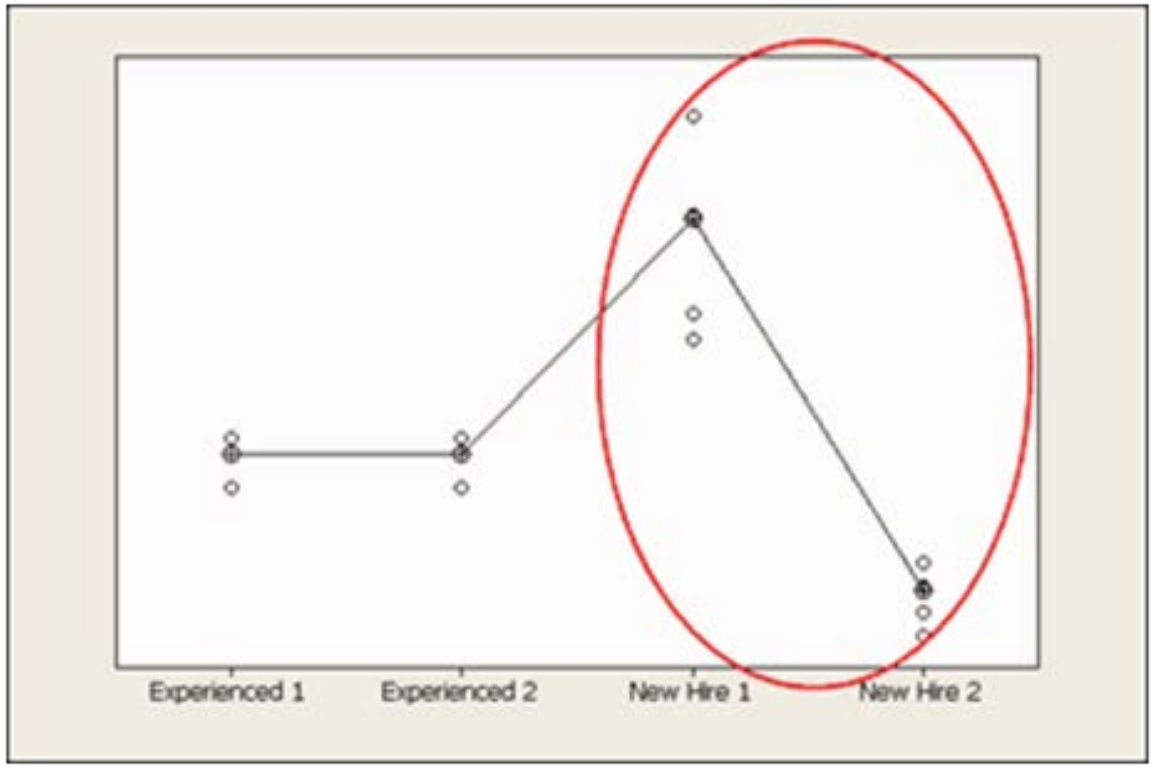
Pipetting Proficiency Test Method

Background

- Pipetting variance can lead to errors which can:
  - Result in unrecognized wrong results
  - Failure to pass a good product (as in QC)
  - Failure to fail a poor product (as in QC)
- Pipetting variance can be attributed to:
  - Poorly seated tips
  - Variable viscosities not taken into account
  - Profusion (residual volume on exterior of tip) carry over
  - Pipette out of calibration (dropped or overly used)
  - Inexperience or need for retraining

Definition

Despite training or prior knowledge, it is possible to drift from uniform practice (as in a golf swing). While pipetting may be effective, it may not yield an accurate and/or consistent result.



Purpose

- This Test Method is designed to characterize pipetting efficiency across the organization.
- Depending on results, there may or may not be a need for training/re-training.
- Uniform pipetting practices are essential to the success of Salimetrics and to our customers.
- This test method, or a revision, is intended to be developed for our customers to help them ensure that their pipetting methods are correct.

Approach

- Who: all individuals who routinely operate pipettes are being asked to participate in this evaluation.
- What: Fluids of varying viscosity will be pipetted by each operator into microwell plates.
- When: within the next 3 days. The procedure should only take a half hour.
- Where: at your own lab bench, with your current pipette
- Why – ibid

Methods

- Fluids of varying viscosities (xanthan gum, water, EtOH) containing yellow dye were developed for this study
- The fluids will be pipetted in multiple replicates by each operator into microwell plates
- All additions are first pass only – no repeats

Single channel pipetting
10 uL (viscous) into dry wells, add 100 uL water, 48 replicates
50 uL (viscous) into dry wells, add 50 uL water, 48 replicates
100 uL (viscous) into dry wells, 48 replicates

Multi channel pipetting
50 uL (viscous) into dry wells, add 50 uL water, 48 replicates
100 uL (viscous) into dry wells, 48 replicates
200 uL (25% EtOH) into dry wells, 48 replicates

Materials

Each operator will be provided:

4 dye fluids made with McCormick's Yellow Dye

- 10 uL fluid (1 mL), dilute dye 58-fold in xanthan gum solution
- 50 uL fluid (8 mL) , dilute dye 291-fold in xanthan gum solution
- 100 uL fluid (15 mL) , dilute dye 580-fold in xanthan gum solution
- 200 uL fluid (15 mL) , dilute dye 1160-fold in 25% EtOH solution

6 uncoated microwell plates

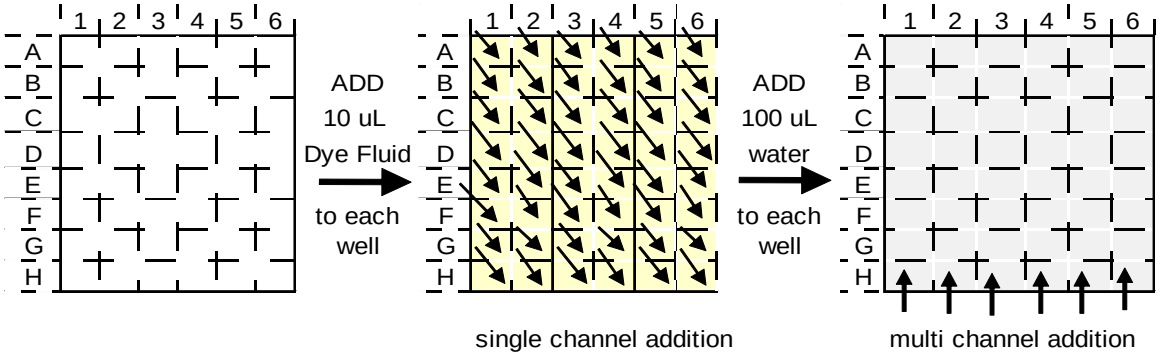
Xanthan Gum Solution
0.95 g/L Xanthan gum in water
0.35 g/L methyl p-hydroxy benzoate (preservative)

25% EtOH Solution
25% EtOH in water
0.35 g/L methyl p-hydroxy benzoate (preservative)

Pipetting Patterns – Single channel

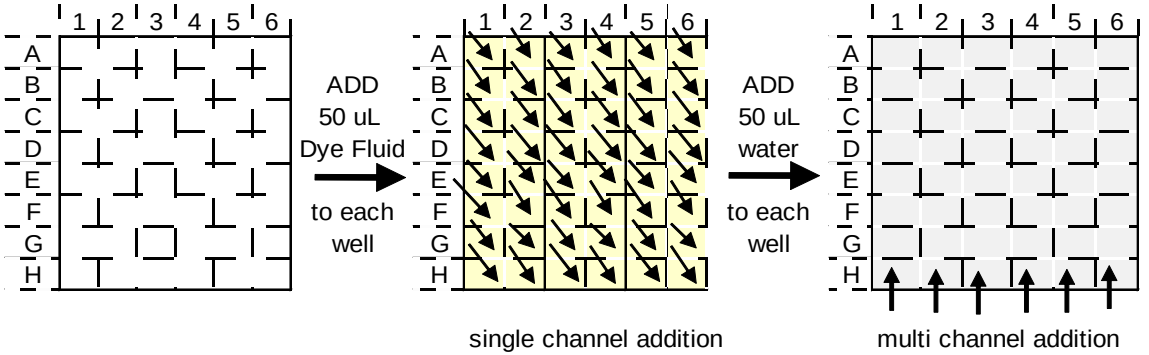
10sc (single channel)

Add 10 uL dye fluid to each of well dry wells.
Add 100 uL water to each column of wells with multichannel pipetter.
Read absorbance at 450 – 492. Export to F: drive



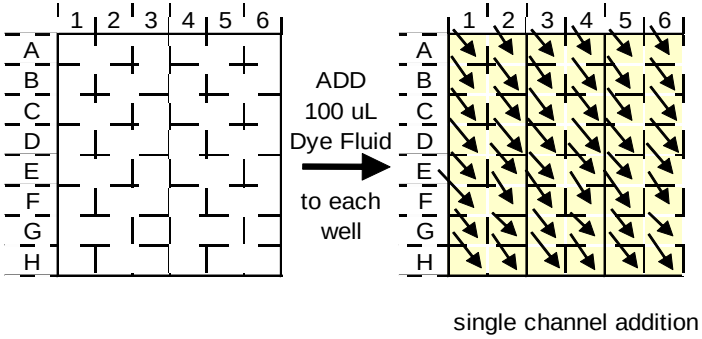
50sc (single channel)

Add 50 uL dye fluid to each of well dry wells.
Add 50 uL water to each column of wells with multichannel pipetter. Read absorbance at 450 – 492. Export to F: drive



100sc (single channel)

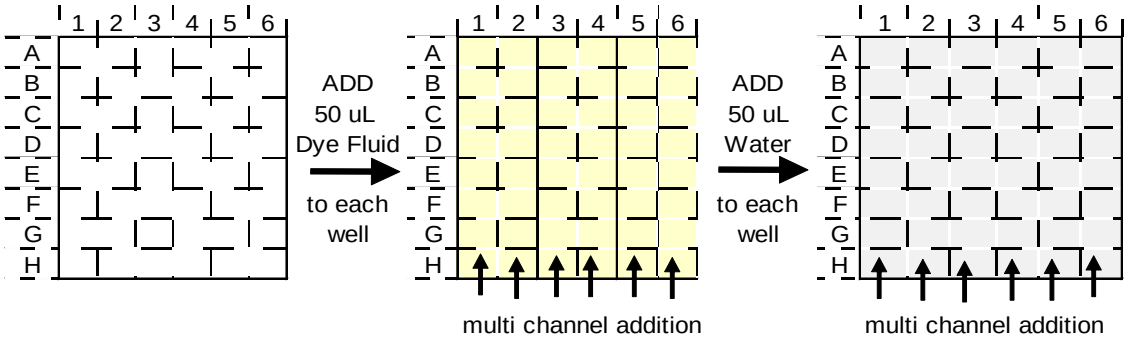
Add 100 uL dye fluid to each of well dry wells.
Read absorbance at 450 – 492. Export to F: drive



Pipetting Patterns - Multichannel

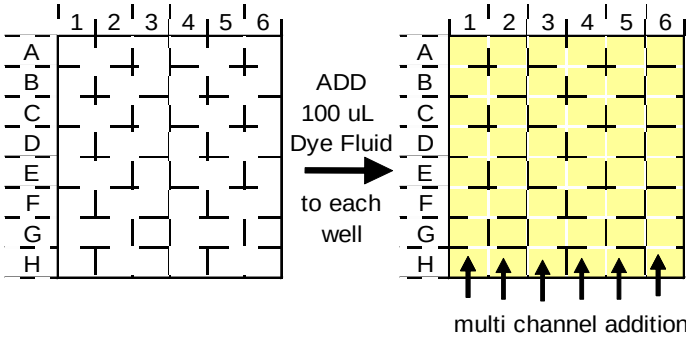
50mc (multi channel)

Add 50 uL dye fluid to each of well dry wells with all eight channels, left to right. Add 50 uL water to each column of wells with multichannel pipetter.
Read absorbance at 450 – 492. Export to F: drive



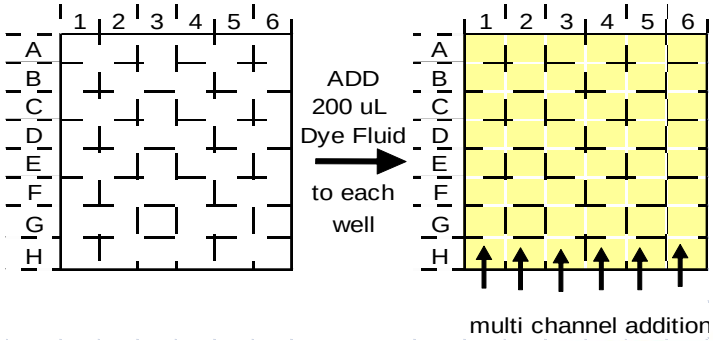
100mc (multi channel)

Add 100 uL dye fluid to each of well dry wells with all eight channels, left to right.
Read absorbance at 450 – 492. Export to F: drive



200mc (multi channel)

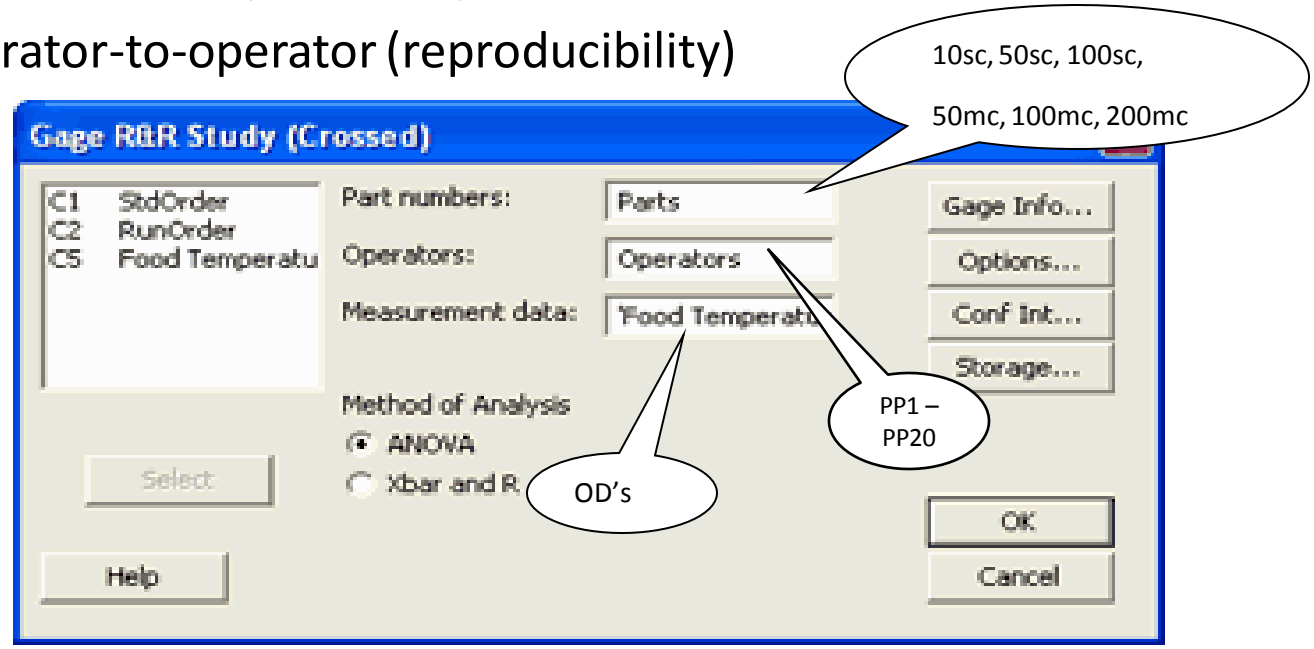
Add 200 uL dye fluid to each of well dry wells with all eight channels, left to right.
Read absorbance at 450 – 492. Export to F: drive



Analysis (GAGE R&R)

The results will be uploaded into MiniTab and analyzed by ANOVA for:

- well-to-well (repeatability)
- operator-to-operator (reproducibility)



The manual and automatic pipetter results may need to be handled separately.

Pipetting Proficiency Evaluation

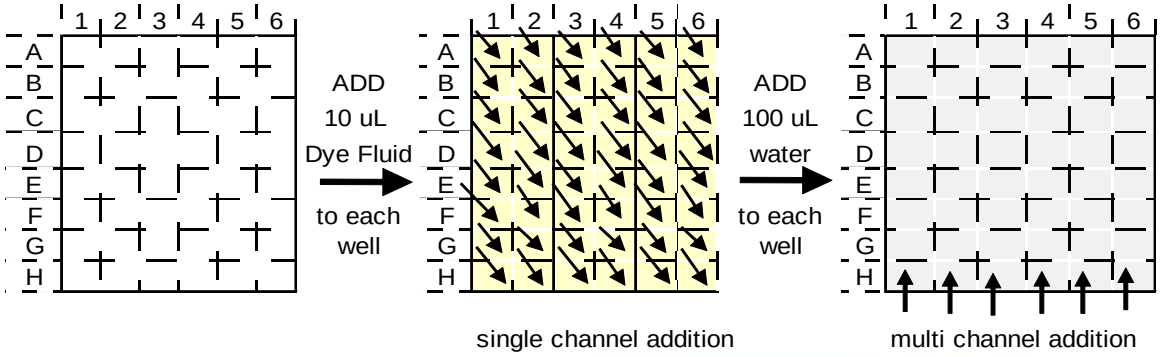
Methods

- Artificial saliva fluids with yellow dye were developed to deliver an OD of 1.0 after pipetting 10, 50, 100 or 200 uL into microwell plates.
- Plates were read on a plate reader and the data uploaded to the f: drive, keeping the identity of the operators anonymous.
- Data was analyzed, by MiniTab: ANOVA, Box/Whisker, Dotplot and GAGE R&R

Example – single channel

10sc (single channel)

Add 10 uL dye fluid to each of well dry wells.
Add 100 uL water to each column of wells with multichannel pipetter.
Read absorbance at 450 – 492. Export to F: drive

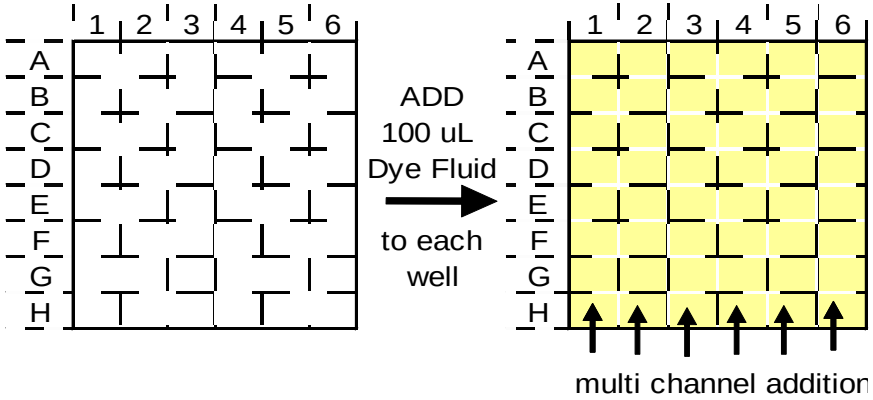


Pipetting Proficiency Evaluation

Example – multi channel

100mc (multi channel)

Add 100 uL dye fluid to each of well dry wells with all eight channels, left to right.
Read absorbance at 450 – 492. Export to F: drive



Results

Thirteen (13) operators each pipetted dye solutions into six plates (48 wells each) for a total of 288 wells

- 10 uL, 50 uL, 100 uL single channel
- 50 uL, 100 uL, 200 uL multichannel

Participants were from QC, Testing, Manufacturing and R&D

10 uL, single channel, worst case fluid,
viscous, high dye concentration, small volume

Within Operator
Avg CV = 8.2%
Stdev = 2.6%

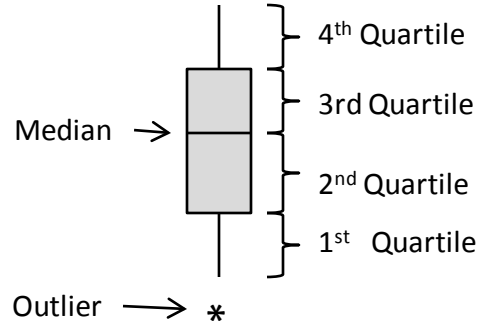
Operator - Operator
CV = 8%

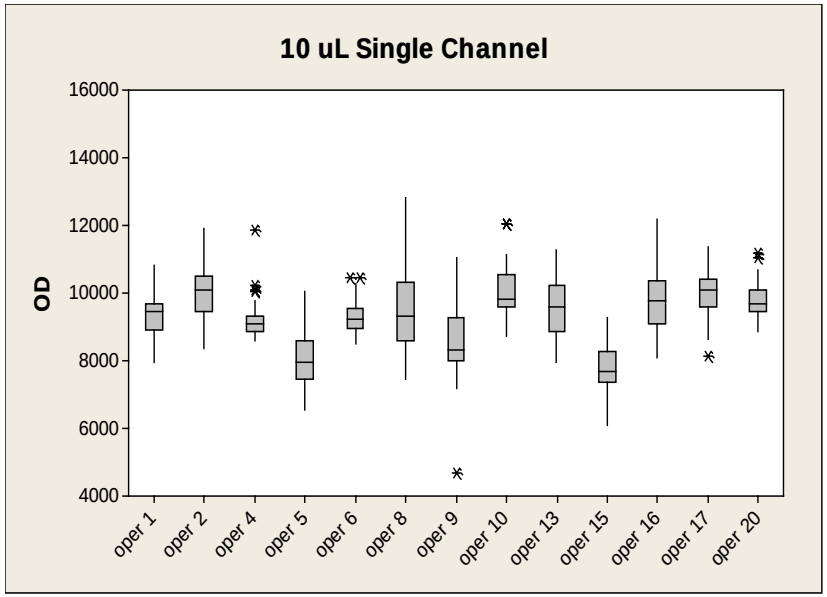
Pooled (Total)
CV = 11%

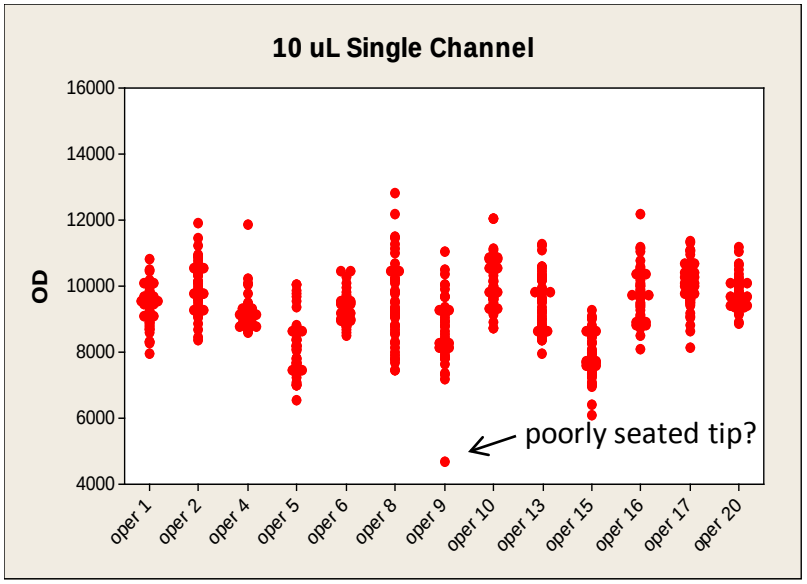
Nested ANOVA:
10 uL SC –vs. Operator, Replicate
Variance Components

Source	
Operator-Operator	46 %
Well-Well	54 %

Box & Whisker Definitions







Example Fluids: Conj – E2, E3, Prog, Testo
Sample – α Amylase, Cort (25), Testo (25)

50 uL, single channel, viscous

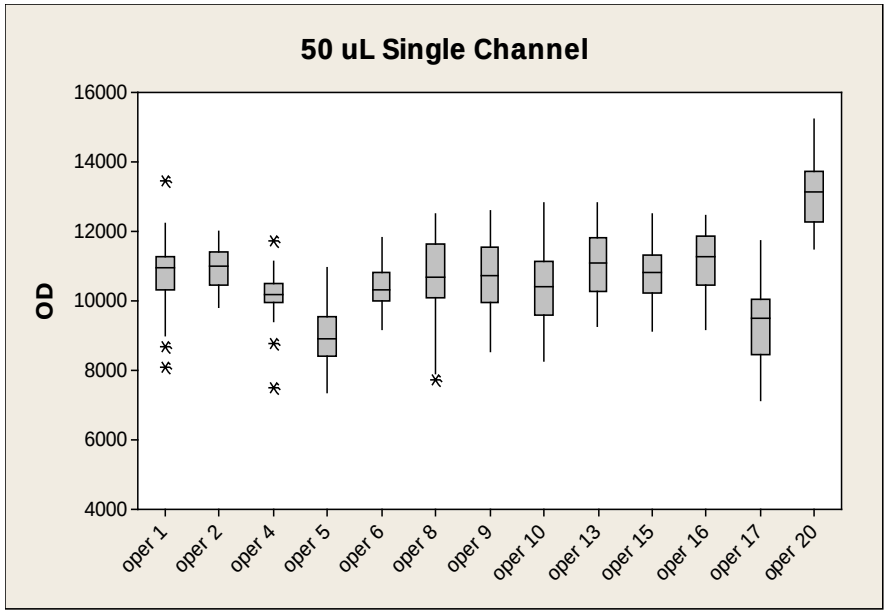
Within Operator
Avg CV = 8.3%
Stdev = 2.0%

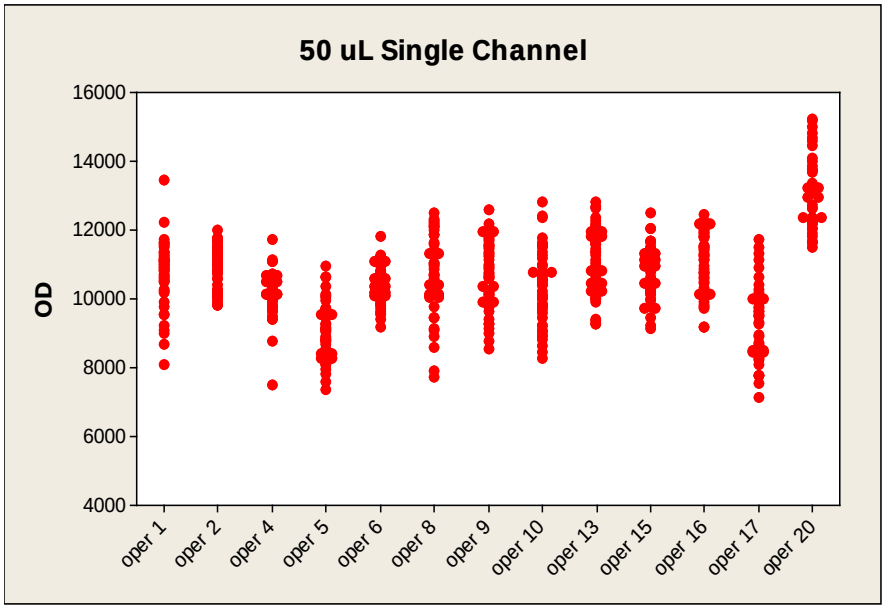
Operator - Operator
CV = 9%

Pooled (Total)
CV = 12%

Nested ANOVA:
10 uL SC –vs. Operator, Replicate
Variance Components

Source	
Operator-Operator	53 %
Well-Well	47 %





Example Fluids : Conj – BC, E1
Sample – Prog, DHEA

100 uL, viscous, single channel

Within Operator

Avg CV =	2.0%
Stdev =	1.0%

Operator - Operator

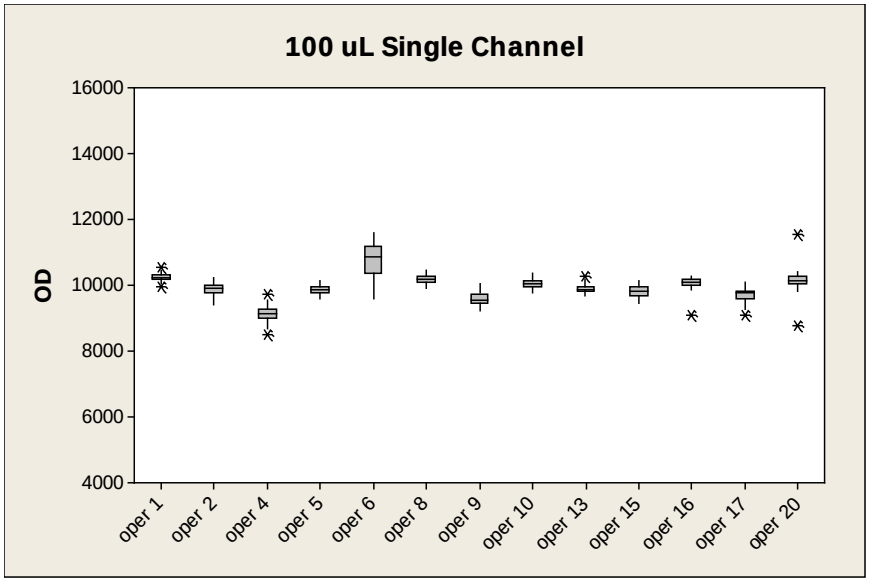
CV =	4%

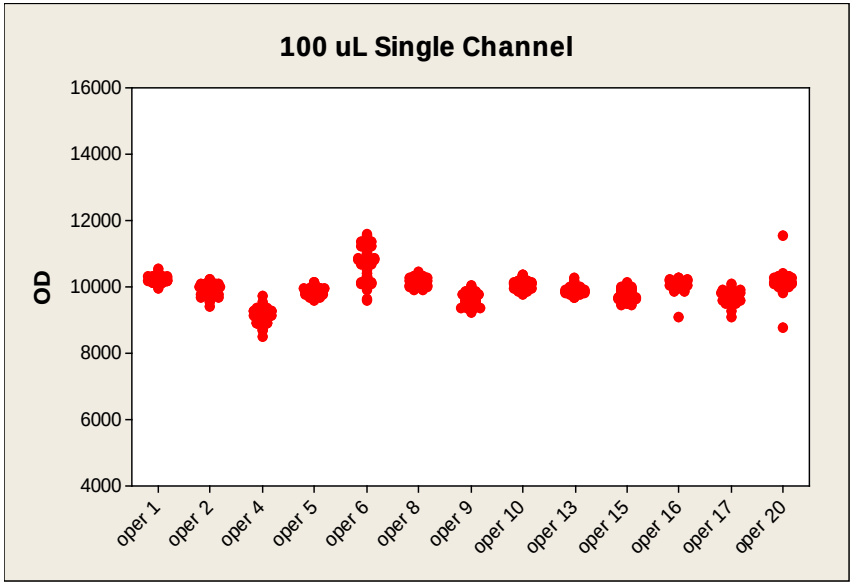
Pooled (Total)

CV =	4%

Nested ANOVA:
10 uL SC –vs. Operator, Replicate
Variance Components

Source	
Operator-Operator	75 %
Well-Well	25 %





Example Fluids : Sample – E1, E2, E3, DHEA-S

50 uL, viscous, multi channel

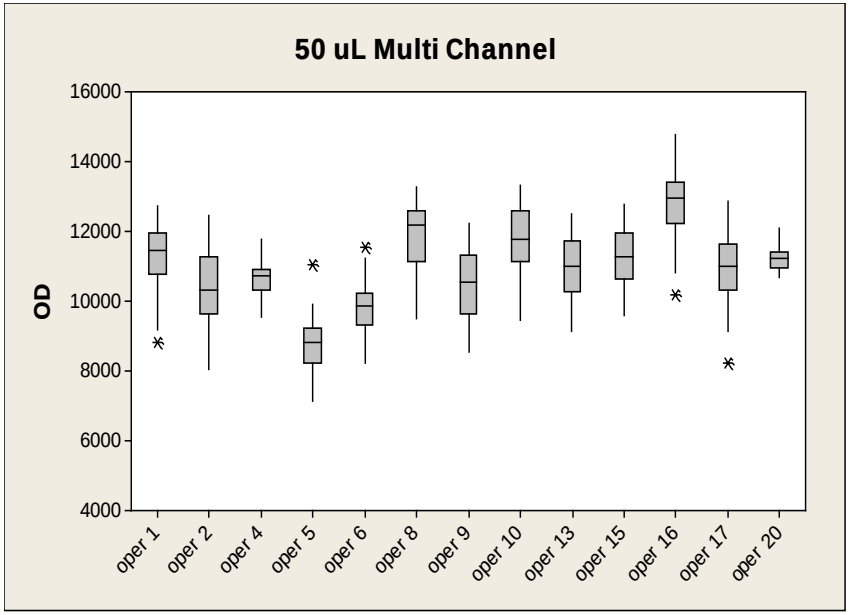
Within Operator
Avg CV = 7.6%
Stdev = 2.2%

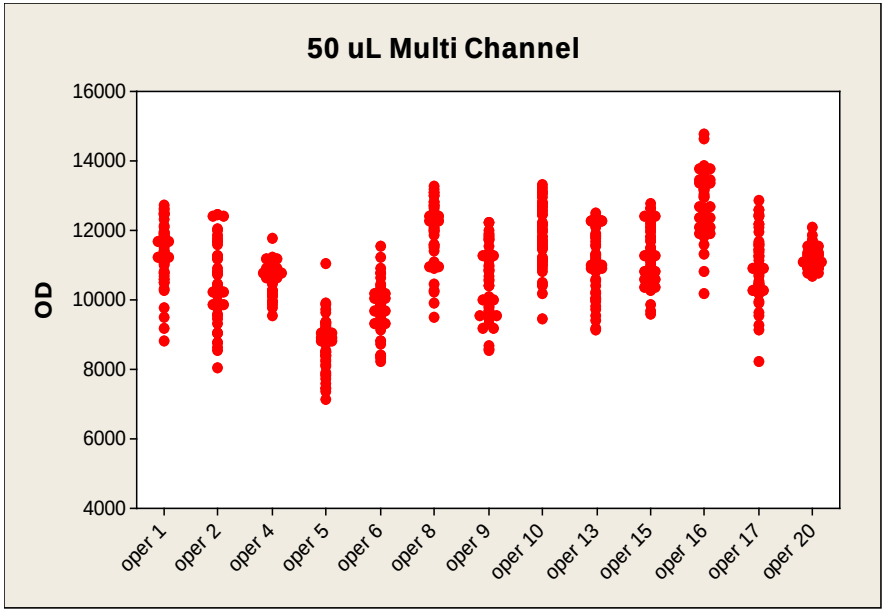
Operator - Operator
CV = 9%

Pooled (Total)
CV = 12%

Nested ANOVA:
10 uL SC –vs. Operator, Replicate
Variance Components

Source	
Operator-Operator	58 %
Well-Well	42 %





100 uL, viscous, multi channel

Within Operator

Avg CV =	1.7%
Stdev =	0.7%

Operator - Operator

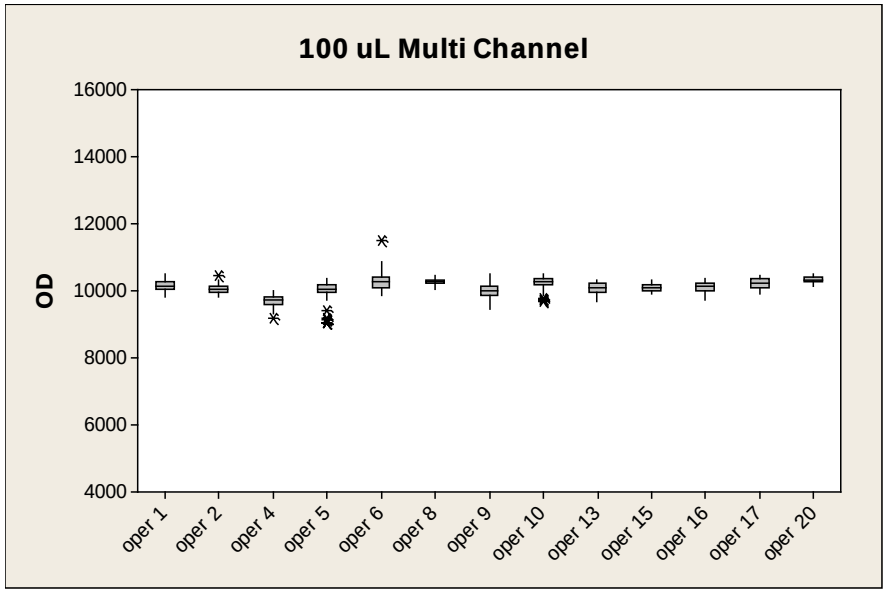
CV =	1.7%

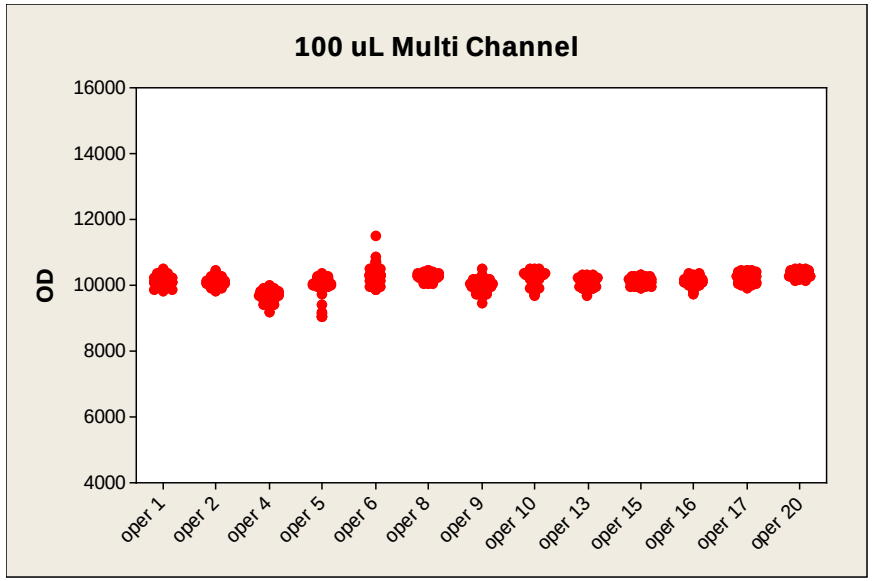
Pooled (Total)

CV =	2.5%

Nested ANOVA:
10 uL SC –vs. Operator, Replicate
Variance Components

Source	
Operator-Operator	44 %
Well-Well	56 %





Example Fluids :
Sample – E1, E2, E3 for HX10 testing

200 uL, low viscosity, multi channel

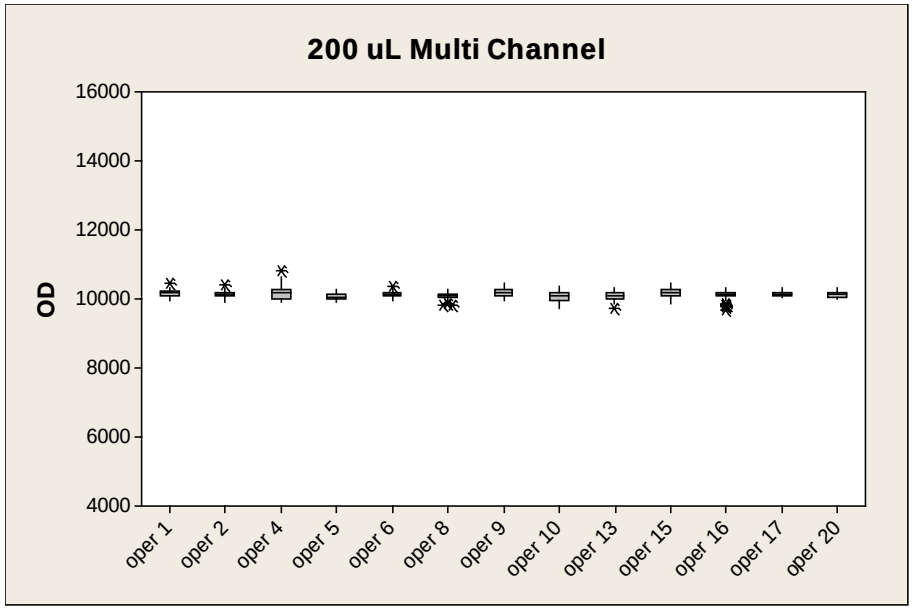
Within Operator
Avg CV = 1.1%
Stdev = 0.3%

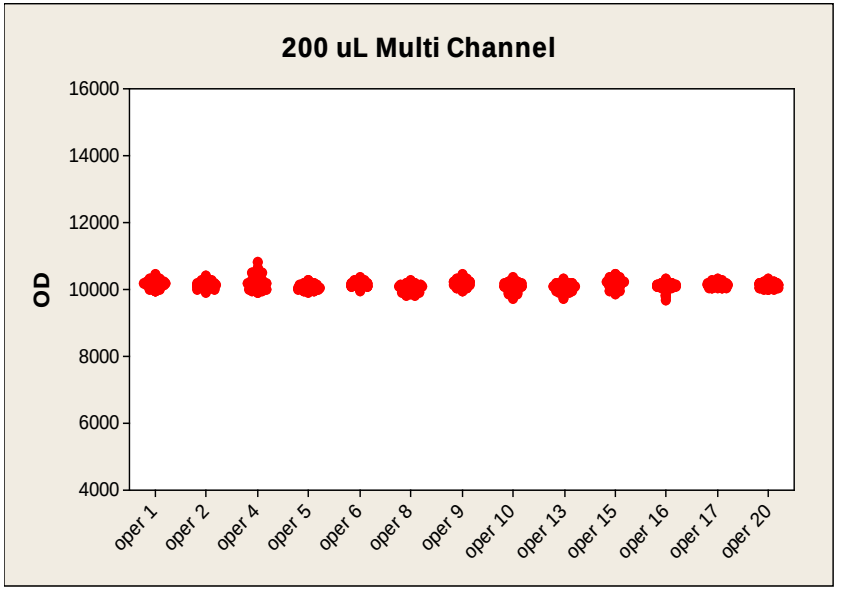
Operator - Operator
CV = 0.5%

Pooled (Total)
CV = 1.3%

Nested ANOVA:
10 uL SC –vs. Operator, Replicate
Variance Components

Source	
Operator-Operator	11 %
Well-Well	89 %





Example Fluid: TMB substrate

Summary and Conclusions

- Levels of error are within expected ranges for manual pipetting.
- Larger volumes typically yield better precision. This is true in automated systems also.
- Pipette types (Hamilton, Finnpipette, Finnpipette 2, BioHit) were not factors (data not shown)
- Testing method was easy to implement, follow and execute.
- Remind operators to ensure tips are well seated and to minimize profusion (already common practice).