Quantifying the Impact of Operator Error in Pipetting

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Abstract

Mechanical action hand-held pipettes are commonly used for transfer of microliter liquid quantities in a variety of analytical laboratories including pharmaceutical quality control, medical testing, forensic analysis, and food safety laboratories. Accurate and precise analytical results from these laboratories are necessary to protect consumers, instill confidence, and support global trade.

Regular calibration of laboratory equipment, including pipettes, is commonly accepted practice in regulated analytical laboratories, but these instrument calibrations are often performed by the OEM or third party services. Such calibrations do ensure that the pipette itself is operating properly, but fail to assess the competence of the pipette operator. In the case of air-displacement mechanical action pipettes, operators are a significant source of pipetting error.

This poster summarizes the significant types of pipetting error using a photometric pipette calibration method. These errors have been observed among "trained and qualified" pipette operators in regulated laboratories, and presents data showing their accuracy and precision performance in routine pipetting.

Data was collected in controlled circumstances to quantify the amount of error that is contributed by each type of operator error. This data leads to a prioritization in operator training which focuses on correcting the specific operator practices that have potential for causing the greatest error in the laboratory.

Introduction

The single channel handheld pipette continues to be one of the most commonly used tools in laboratories. The frequency and ease of use means their dependability often goes unquestioned. However, like many analytical instruments, much of the reliability of pipetting can be contributed directly to user technique. Training and skill assessment play a major role in the success of an operator and the reliability of data. Yet even with proper training, it is easy to fall into habits that can be detrimental to the end result.

Overview of the Experimental Methods

The standard pipetting method used in this experiment is as follows:

- Volume measurements were made using the photometric method in accordance with ISO 8655-7 using the ARTEL PCS® Pipette Calibration System. Technicians pipette a red dye sample solution into a vial containing blue dye. The PCS® accurately measures dispensed volume
- The pipette tip was prewet by aspirating and dispensing an aliquot of the sample solution three times
- With the plunger depressed to the first stop, the tip was immersed 2 millimeters into the sample solution. Each tip was marked at 2 mm
- The aliquot of red dye was aspirated from the sample solution by gently releasing the plunger, keeping the tip in the sample solution for one second before removal
- The aliquot was delivered into the blue dye by placing the pipette tip on the side of the glass vial at a 45° angle just above the meniscus and slowly depressing the plunger to the first stop, then to the second stop, delivering the entire aliquot
- Each experiment consisted of two runs of thirty data points each, using an adjustable 20 µL (Pipette A) and 100 µL (Pipette B) manual action air displacement pipette set at 20.00 µL and manufacturers recommended disposable pipette tips
- Each experiment was performed as a comparison of one pipetting technique versus another using the standard pipetting method as the control. The results for each of the techniques were evaluated for precision and accuracy
- The measurement used for rating the precision of each technique is the coefficient of variance (CV). Inaccuracy (RI) is defined as the percent difference in mean delivery volumes versus the volume indicator on the pipette
- All experiments were carried out in a well controlled laboratory environment. Temperature was maintained at 20.0 ±1.0 C; relative humidity was maintained at 45-65%

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What is Imprecision and Inaccuracy?

Imprecision is concerned with the closeness of two or more measurements to each other, measured in terms of standard deviation or as coefficient of variation CV.

Inaccuracy is the deviation of a measurement from a standard value or true quantity. In a group of values, the deviation of the mean from a standard value.

Common pipetting errors:

- **Pipette tips not equilibrated to the test environment**
- **Reverse mode pipetting**
- **Dragging tip while exiting solution**
- **Immersion depth**
- **Aspiration rate**
- **Wiping pipette tip with absorbent material before delivery**
- **Pause timing during aspiration**
- **Hand warming**
- **Generic tips**

Results

Figure 1. Imprecision of pipette A, a 20 µL pipette set to 20 µL

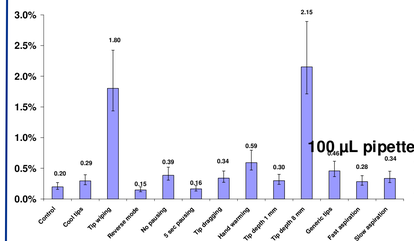


Figure 2. Inaccuracy of pipette A, a 20 µL pipette set to 20 µL

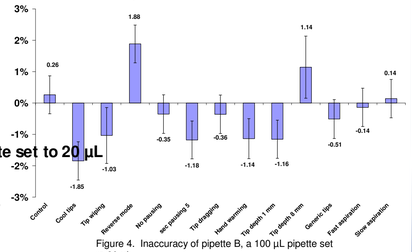


Figure 3. Imprecision of pipette B, a 100 µL pipette set to 20 µL

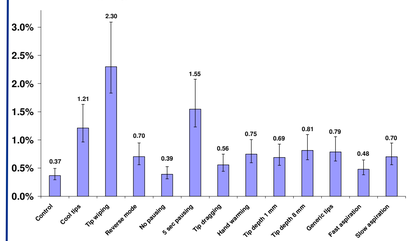
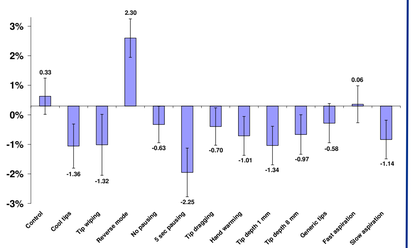


Figure 4. Inaccuracy of pipette B, a 100 µL pipette set to 20 µL



Analysis

Including the control case, a total of 13 calibrations were performed for each pipette. The measured relative inaccuracy (RI) and imprecision (CV) are summarized in the bar charts.

Linear interpolation was used to establish an expected manufacturer's specification for the 100 µL pipette set to deliver 20 µL.

For the 20 µL nominal volume pipette the CV spec is 0.30%. For this pipette the control case is clearly within specification. Two other cases also produced CV results within specification (reverse mode and 5 second pausing). There are five cases where the pipette clearly performed outside of the manufacturers CV specification. These are: 8 mm immersion, tip dragging, hand warming, generic tips, and no pausing. Finally, there are five other cases where conformance with the CV specification was indeterminate (i.e. the confidence interval for the CV result straddles the specification). See Figure 1.

The RI specification for the 20 µL nominal volume pipette is ±1.00%. The control case is clearly within specification, and four of the 12 cases also produce results within specification. These are: no pausing, tip dragging, fast plunger, and slow plunger. There are two cases outside manufacturer's specification- reverse mode and cool tips. The remaining six are indeterminate. See Figure 2.

For the 100 µL nominal volume pipette set at 20 µL the CV spec is 0.56%. The control case is clearly in specification, with one other (no pausing). There are seven cases were CV fell out of specification. These are (tip wiping, 5 second pausing, cool tips, 8 mm immersion, generic tips, hand warming, and reverse mode pipetting. The remaining four are indeterminate. See Figure 3.

The RI specification for the 100 µL nominal volume pipette set at 20 µL is ±1.75%. The control case is clearly in specification, along with eight of the 12 experimental cases. These are: no pausing, tip dragging, hand warming, 8 mm immersion, 1 mm immersion, generic tips, fast plunger, and slow plunger. The three other cases are indeterminate. One case, reverse mode pipetting, is clearly out of tolerance. See Figure 4.

Table 1. Summerized data for both pipettes, all experiments

	100 µL pipette set to 20 µL		20 µL pipette set to 20 µL	
Test	**Imprecision**	**Inaccuracy**	**Imprecision**	**Inaccuracy**
Control	0.37%	0.33%	0.20%	0.26%
Cool tips	1.21%	-1.36%	0.29%	-1.85%
Reverse mode	0.70%	2.30%	0.15%	1.88%
Tip Dragging	0.56%	-0.70%	0.34%	-0.36%
Immersion depth 1 mm	0.69%	-1.34%	0.30%	-1.16%
Immersion depth 8 mm	0.81%	-0.97%	2.15%	1.14%
Slow plunger release	0.70%	-1.14%	0.34%	0.14%
Fast plunger release	0.48%	0.06%	0.28%	-0.14%
Tip wiping	2.30%	-1.32%	1.80%	-1.03%
Pausing 5 sec.	1.55%	-2.25%	0.16%	-1.18%
No pausing	0.39%	-0.63%	0.39%	-0.35%
Hand warming	0.75%	-1.01%	0.59%	-1.14%
Generic tips	0.79%	-0.58%	0.46%	-0.51%

Conclusions

Minor variations in operator technique can produce measurable differences in the accuracy and precision of the handheld air displacement pipettes. Reverse mode pipetting consistently produced large positive accuracy errors, while tip wiping produced negative shifts in accuracy and large CVs. This finding supports the need for standardization of operator technique within a laboratory, followed by training and testing to ensure that pipette operators have achieved the degree of equivalence necessary for a given laboratory application.

The combinations of errors can impact accuracy and precision by as much as 12%. The trained operators in this study were varying one technique at a time. However, it is common to see multiple simultaneous variations in the field. It is likely that errors in the field are even larger than observed in this study. To reduce pipetting errors to obtain accurate and precise results follow these important steps:

- **Prewet the pipette tip**
- **Work at temperature equilibrium**
- **Use standard mode pipetting**
- **Pause consistently after aspiration**
- **Hold pipette vertically when aspirating**
- **Immerse pipette tip to the proper depth**
- **Use the correct pipette tip**
- **Use consistent plunger pressure and speed**

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