Achieving Optimum Accuracy with Adjustable Volume Pipettes

Air-displacement pipettes are among the most commonly used lab tools. Given most scientists' familiarity with these instruments, the selection of the right pipette for any particular task may seem obvious. Yet our experience is that while many scientists want the most accurate pipettes, many also misunderstand a key specification, and that misunderstanding can compromise their results. Specifically, it is vital to understand how "accuracy" specifications are calculated to select the pipette for any particular task that will provide the most reliable results.

WHAT IS AN "AIR-DISPLACEMENT" PIPETTE?

Most common laboratory pipettes (single- and multi-channel, mechanical, and electronic) are air-displacement pipettes. They are so-named because they operate by having a piston of consistent diameter pushed through a seal to move a column of air. The movement of the air column, in turn, moves the liquid to be measured into and out of a disposable polypropylene tip.

To aspirate once the tip is attached, the piston is moved forward from its resting position through the seal to push air out of the pipette. The tip is placed into the liquid to be aspirated and the piston is allowed to move backwards, powered by a low force spring. This creates a vacuum in the pipette tip and atmospheric pressure pushes the liquid to be pipetted into the tip, much as a U-tube manometer responds to pressure differentials.

Adjustable volume pipettes work with the assumption that the piston is completely uniform so that the relationship between the distance traveled by the piston and the volume of air (and thus, liquid) moved is linear. To adjust the pipetted volume, a screw mechanism is used to adjust precisely the distance the piston travels; cut the distance traveled in half, and you cut the pipetted volume in half, and so on.

HOW ACCURATE ARE AIR-DISPLACEMENT PIPETTES?

For the most common volumes handled by adjustable-volume microliter pipettes (from about 50 µL and higher), most of the inaccuracy of the instrument can be thought of as a constant volume[1] that applies through the entire volume range of the instrument. This volume of inaccuracy is a constant regardless of the volume setting on the instrument.

Table 1. Volume of inaccuracy of a 1,000-µL adjustable volume pipette with "1.0% accuracy" at nominal volume

Volume Setting(µL)	Percent of Nominal Volume	Volume of Inaccuracy (µL)	± % Inaccuracy
1,000	100	10	1.0
900	90	10	1.1
500	50	10	2.0
200	20	10	5
100	10	10	10
50	5	10	20

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On the other hand, when the "accuracy" [2] of an instrument is shown in catalogs and other literature, it is often displayed as a percentage. The percentage is based on the nominal capacity, or maximum volume, of the instrument. This one specification is responsible for a lot of misunderstanding. As noted above, the instrument's volume of inaccuracy remains constant through the volume range, so that fixed volume of inaccuracy represents a larger percentage inaccuracy for lower volume settings on the same pipette (Figure 1). For example, a pipette with published "1% accuracy" at full scale translates to a 2% inaccuracy at 50% of full scale, and a 10% inaccuracy at 10% of full scale (Table 1).

Because of this phenomenon, the most accurate measurements will be made when operating a pipette as close to the nominal capacity (maximum volume) as possible. In fact, when different pipette manufacturers choose to specify a lower volume limit of 5% or 10% of maximum scale, the choice may have little to do with the quality of the pipette or the accuracy at those low volumes, and more to do with the manufacturer's perception of what constitutes "acceptable" inaccuracy in scientific work.

WHICH PIPETTE FOR WHICH TASK?

Most labs need to pipette a wide range of liquid volumes and are equipped with a set of pipettes that cover the volume range. Suppose you have three pipettes with nominal capacities of 1–10 µL, 10–100 µL, and 100–1,000 µL, respectively, and that each pipette has a specification of 1% accuracy. Based on the discussion above, we know that "1% accuracy" refers to the volume at nominal capacity, and that at the bottom of the range, the volume of inaccuracy will be the same, and the percent inaccuracy ten times as high.

Now imagine a case in which you need to pipette 200 µL. You have three choices. You can rely on the 1,000-µL pipette, and know that you will have an accuracy of ±10 µL, or 5% at this volume. You can select the 100-µL pipette, and have an accuracy of ±1 µL (1%), but have to pipette twice (for a total inaccuracy of ±2 µL) to achieve the desired volume. Your third choice is to buy a new pipette with a nominal volume (or fixed volume) of 200 µL, with ±1% accuracy (2 µL). Any one of these choices may be acceptable, depending on your budget and need for accuracy and productivity, but it is critical to understand the choice you are making so that you make the right choice for your lab.

The illustration is simplest, as above, when one option is to purchase a pipette with the exact volume you need. In most cases, the desired volume falls somewhere within the volume ranges of several pipettes. For example, 20 µL can be pipetted with 2–20 µL, 5–50 µL, or 10–100 µL pipettes. We know that the highest accuracy will be achieved with a 2–20-µL pipette, but if you don't have such an instrument, it will always be more accurate to choose instrument with the lowest volume relative to your aliquot volume. In this case, the 5–50 µL will be a better choice than the 10–100 µL.

EQUIPPING A NEW LAB

In view of the foregoing discussion, one can equip a lab with a range of pipettes that just cover the anticipated pipetting range. In such a case, it is critical to realize that the accuracy of the work in that lab will vary greatly depending on whether a particular sample can be pipetted at the top, middle, or bottom of the ranges of the available pipettes. If budget permits, the accuracy can be made much more consistent (and, typically, better) by having a set of pipettes that overlap in their volume ranges. For example, if your lab has pipettes of 1–10 µL , 10–100 µL, and 100–1,000 µL , equipping a lab with just two more pipettes of intermediate volumes (say, a 5–50 µL and a 20–200 µL) will ensure that many more procedures can be performed at accuracies close to the manufacturer's percent accuracy specification for your pipettes. Without these two additional pipettes, a lab may be forced to run some tests that risk inaccuracies of up to ten times the nominal volume specification, or to pipette repeatedly using a smaller volume pipette.

NOTES

1. *Inaccuracy is the difference between the actual pipetted volume and the intended volume. Since this value is calculated as an average, the actual volume of inaccuracy in individual pipetted volumes varies. For this discussion we are referring to the specification represented by the average inaccuracy. Smaller, but significant, contributors to manufacturers' inaccuracy specifications are the precision of manufacture and — in volumes below 50 µL — factors such as the compressibility of air and capillary action. Outside of the optimal conditions of the manufacturer's calibration lab, user technique and the quality of tips used are also significant contributors to inaccuracy.*

2. *The "accuracy" specification used to describe a pipette is technically the accuracy tolerance, that is, the absolute value of the anticipated deviations from the intended volume. In this article, since we are repeatedly referring to that volume error, we refer to the "inaccuracy" of the pipette except where we refer to published specifications.*

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