Best Practices for the Use of Micropipettes
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Introduction

Mechanical action micropipettes are ubiquitous in laboratories and are used for many routine tasks, including the quantitative measurement and dispensing of analytical samples and reagents. As concentrations of biological and chemical components in the prepared samples for analyses and assays are volume-dependent, improperly performed pipetting steps will directly impact the transferred volumes, and hence, the test results. The design and construction of piston-operated air-displacement pipettes (in this paper simply referred to as pistoles) render their performance susceptible to the pipetting technique and skills used by the operator of such devices.

This study evaluates a number of techniques that influence the accuracy and precision of the pipetted volume. The pipette operator has the ability to control all of these parameters by using the appropriate pipetting technique, as well as by choosing the appropriate pipette size and type of pipette tip.

Influence of the Air Cushion on Pipette Performance

Piston-operated air-displacement pipettes use an air cushion to couple the plunger of the pipette to the aspirated liquid inside of the pipette tip. This air cushion, often referred to as captive air volume or dead air volume, is trapped within the pipette as soon as the tip is immersed in the sample solution. This captive air volume closely follows the Ideal Gas Law (PV = nRT, where P is the pressure of the trapped gas, V is the volume, n is the number of moles and T is the temperature of this gas).

The ideal gas law allows for the estimation of the effects which temperature, evaporation, and the ratio of captive air volume to the pipette’s dead space volume will have on the actually aspirated and delivered volume of a pipetting cycle. The following techniques studied here directly influence the captive air volume: Pre-wetting of Pipette Tips, Temperature Dis-equilibrium, Hand Warming, and Immersion Depth of Pipette Tip.

Position of Tip during Aspirating and Dispensing

In order to ensure the optimum and undisturbed hydrodynamic flow of the sample solution during aspiration of the sample into the tip, the pipette should be held in a vertical position, and the tip should not touch the side or bottom of the vessel with the sample liquid. Further, it is important not to drag the tip along the wall of the source sample vessel, as this may lead up to -0.7% RI and 0.6% CV.

When dispensing the sample using forward mode, it is recommended to touch the pipette tip against the side of the receptacle, while the pipette may be held at a 45° angle. With the exception of pipetting very small volumes, it is not recommended to immerse the tip into already present solution in the receiving vessel. This technique may lead to over-delivery if droplets are clinging to the inside of the pipette tip, and significantly increases the risk of cross-contamination.

Pause after Aspirating

Once the aliquot of sample solution has been aspirated into the pipette tip, it is important to pause for about 1 second with the tip still immersed in the sample liquid, allowing the sample to “settle” in the tip. Removing the pipette tip prior to allowing the vibrational motion of the liquid to settle will introduce errors in the precision and accuracy, up to -0.6% RI and 0.4% CV in our studies. Allowing the tip to remain in the liquid for too long, however, will result in significant under-delivery, up to -2.3% RI and 1.8% CV. The magnitude of these errors depends on the pipette tip, temperature, sample type (vapor pressure), speed of aspiration, and the sample volume.

Tip Wiping

The practice of wiping the pipette tip after aspiration with an absorbent laboratory cloth is a wide-spread habit. Due to the high propensity of introducing large errors through this technique, it should be very carefully evaluated whether this is really necessary. If it is determined that a particular sample is prone to forming droplets on the outside of the pipette tip that need to be wiped off, extreme care should be exercised in not touching the tip orifice, as it is very easy to wipe out some of the sample solution through the tip orifice by moving the tissue in its close vicinity. Despite exercising great care when wiping off the pipette tip in our study, it introduced over 2.3% CV and consistently led to under-delivery of up to -1.3% RI.

Pipette Tip Quality

For the most accurate and precise pipetting results, the pipette manufacturer’s recommended tips should be used. Achieving a proper seal between the pipette’s nose cone and tip is critical for good performance. Generic tips may seemingly fit on a pipette, but due to different taper angles of the nose cone and tip, a poor seal is established, resulting in errors. In our study, the generic tips fit on the pipettes but still introduced errors of up to -0.6% RI and 0.8% CV, which would be additive to all other pipetting errors.

Claimed pipette performance assumes the use of manufacturer’s recommended tips. Regardless of the type of tip used in the lab, it is imperative that a pipette is calibrated with this very same tip type and under the conditions of its use in the lab in order to avoid errors while using the pipette for analytical tests.

Pipette Size

Adjustable volume pipettes can be used over a large range of volumes. Manually operated pipettes usually allow the user to select volumes as low as 10% of the pipette’s nominal volume. Several electronically operated pipettes offer an even wider range of selectable volumes. Best pipette performance, however, is achieved at or near the nominal volume of a pipette. For best results, it is recommended to use variable-volume pipettes only to the nominal volume of the next available, smaller denomination of pipette.

Experimental Conditions

• All tests reported in Figures 2 to 5 were conducted in a controlled calibration laboratory, at 20.0 ± 1.0 °C and 45-50% relative humidity.
• Volume measurements were performed with an Artel PCS® Pipette Calibration System, using the photometric method according to ISO 8659-7.
• Only one parameter of the pipetting technique was varied in each experiment and compared to the control method described below; compounding of technique errors was not investigated in this study.

• Two pipettes were evaluated in this study:
  (1) 20 µL pipette set at 20 µL
  (2) 100 µL pipette set at 20 µL
• The manufacturer’s recommended tips were used.

• Experiments were carried out by trained operators.
• The control method used the following pipetting technique:
  (1) each pipette was pre-wetted 3 times
  (2) the tip was immersed 2 mm below the meniscus in the sample solution;
  (3) pipette was held in a vertical position during aspiration, and at a 45° angle during the dispense against the glass wall of the measurement cuvette;
  (4) forward mode of pipetting was used, with blow-out during dispensing.
• Each experiment was conducted with 30 replicates.
• A new pipette tip was used for each data point.
• Accuracy is reported as Relative Inaccuracy (RI) as percent difference to the set volume of the pipette.
• Precision is reported as the Coefficient of Variance (CV).

References

(3) Cate, A.B. Dry Heat and Humidity - Lab-Evironmental Conditions as Sources of Error, Lab Business 2009, online at http://www.labbusinessmag.com/articles/winter09dryheat/dryheat.html

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