Best Practices for the Use of Micropipets

Every day, air-displacement pipets are used to quantitatively dispense sample and reagent aliquots for reaction, routine analyses, and specialized tests. Since concentrations of biological and chemical components in the prepared samples for analyses and assays are volume-dependent, incorrectly performed pipetting steps will directly impact the transferred volumes, and hence, the test results. The design and construction of piston-operated air-displacement pipets render their performance susceptible to the pipetting technique and skills used by the operator of such devices. The pipet operator usually has the ability to mitigate the influence of most parameters by using the appropriate technique, as well as by choosing the appropriate pipet size and type of pipet tips.

Influence of the air cushion on pipet performance

Piston-operated air-displacement pipets use an air cushion to couple the pipet’s piston to the aspirated liquid inside of the pipet tip. This air cushion, often referred to as captive air volume or dead air volume, is trapped within the pipet as soon as the tip is immersed in the sample solution. This captive air volume closely obeys the Ideal Gas Law ($P_a V_a = n_a R T_a$).

The Ideal Gas Law allows one to estimate the effects that temperature and, by extension, evaporation, and the ratio of captive air volume to the pipet’s set 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: prewetting of pipet tips, temperature disequilibrium, hand warming, and immersion depth of pipet tip.

Since the total volume of the air cushion can vary widely depending on the type of pipet, the tip type and size, and the amount of the aspirated liquid aliquot, this study evaluated two different scenarios: one set of experiments was conducted with a 20-μL pipet set at 20 μL, and the other experiments with a 100-μL pipet set at 20 μL.

Prewetting of pipet tips

Sample solution in the pipet tip is susceptible to evaporation into the air cushion during and after aspiration. The evaporative loss of sample solution is dependent on the humidity of the captive air space, as well as the temperature of the sample solution. Repeated aspiration/dispense cycles will increase the humidity of the air in the pipet tip and shaft. Figure 1 shows the dispensed volumes of a 20-μL pipet set to 20 μL and used in a noncontrolled laboratory (30% relative humidity). Each dispense was performed with a new tip. Without prewetting the tips, the pipet dispensed on average 1.3% less volume, as compared to dispenses when the tip was prewetted three times prior to the dispense. When using pipets in particularly dry or warm environments, the error can be significantly larger without prewetting the tips.

Temperature disequilibrium

For most accurate pipetting results, it is recommended that the pipet, the pipet tip, and the sample solution have been equilibrated for at least 2 hr and are within 0.5 °C of ambient temperature. Many samples, however, must be handled at specific high or low temperatures, and pipetting such samples can introduce significant errors in the delivered volume due to the expansion or contraction of the captive air volume and evaporation. Studies of this effect have been reported previously.

The present study (see experimental conditions) evaluated the use of pipet tips that had been cooled to 4 °C for 30 min prior to use. Pipetting with these cold tips led to significant underdelivery of sample with both pipets, contributing up to −1.9% relative inaccuracy (RI) and 1.2% coefficient of variance (CV) to the errors. The inaccuracy and imprecision results for both pipets and all tested scenarios described here are graphed in Figures 2–5. Experimental conditions are shown in Table 1.

Heat transfer/hand warming

Handling a pipet for prolonged periods of time will cause the barrel of the pipet to warm, leading to an expansion of the captive air volume, ultimately impacting the accuracy and precision. Progressive warming of the pipet’s barrel through heat transfer from the hand manifests itself by a trend toward smaller delivered volumes, and led to −1.1% RI and 0.8% CV in this study.
Immersion depth of the pipet tip

Immersing the pipet tip to the proper depth during aspiration of the sample is important. Pipet calibration standards like ASTM E1154 recommend an immersion depth of 2–3 mm for pipet volumes of 1–100 μL, 2–4 mm for 101–1000 μL, and 3–6 mm for volumes larger than 1 mL. In this study we evaluated immersion depths of 1 mm and 8 mm. A shallow immersion depth increases the risk of aspirating small amounts of air, while immersing tips too deeply increases the risk of carrying over droplets on the outside of the tip, and/or forcing more sample in the tip due to increased hydrostatic pressure on the outside of the tip. Either case leads to a significantly increased imprecision (up to 2.2% CV) of the delivered volumes.

Forward and reverse mode

Use of the appropriate pipetting mode has one of the biggest influences on the accuracy of the volume delivery. In forward mode, the plunger is depressed to the first stop, the pipet tip is then immersed in the sample, and the sample is subsequently aspirated. During delivery, the plunger is depressed beyond the first stop (blow-out stop), forcing all the liquid out of the tip. Standard procedure for pipet calibration prescribes using this forward mode and aqueous sample solutions.

In reverse mode, the plunger is depressed beyond the first stop (to the second stop) before immersing the tip in the sample, aspirating more...
than the desired sample volume. The desired volume is delivered by de-
pressing the plunger to the first stop, retaining the additional sample in
the tip. While this pipetting mode is recommended for use with viscous
or volatile solutions, using reverse mode with aqueous solutions leads to
significant overdelivery of up to 2.3% RI and contributes up to 0.7% CV.

Consistent plunger speed and pressure
Depressing and releasing the plunger with consistent speed during
aspiration and dispensing of the liquid aliquot is important for achieving
precise and accurate results. The type of pipet, tip, and sample solution
will determine the optimum pressure needed to move the plunger
with a consistent and appropriate speed. Our studies indicate that a
slow aspiration speed may result in underdelivery of up to ~1.1% RI and
contribute up to 0.7% CV.

Position of tip during aspirating and dispensing
Holding the pipet in a vertical orientation and preventing the tip
from touching the side or bottom of the sample vessel will ensure an
optimal and undisturbed hydrodynamic flow of the sample during
aspiration. Further, it is important not to drag the tip along the wall of
the source vessel after aspiration, because this may lead up to ~0.7% RI
and 0.6% CV.

When dispensing the sample, it is recommended to touch the pipet
tip against the side of the receptacle, while the pipet 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, because this may lead to overdelivery if droplets are
clinging to the outside of the tip, and significantly increases the risk of
cross-contamination.

Pause after aspirating
Once the aliquot of sample solution has been aspirated into the pipet
tip, it is important to pause for about 1 sec with the tip still immersed in
the source liquid, allowing the sample to “settle” in the tip. Removing
the pipet 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 underdelivery, up to ~2.3%
RI and 1.6% CV. The magnitude of these errors depends on the pipet
tip, temperature, sample type (vapor pressure), speed of aspiration, and
sample volume.

Tip wiping
The practice of wiping the pipet tip after aspiration with a laboratory
cloth is widespread. Due to the high propensity of introducing large
errors through this technique, one should be very carefully evaluate
whether this step is really necessary. If it is determined that a particular
sample is prone to forming droplets on the outside of the pipet tip that
must be wiped off, extreme care should be taken not to touch the tip or-
ifice, since it is very easy to wick out some of the sample solution. In our
study, tip wiping introduced over 2.3% of CV and led to underdelivery of
up to ~1.3% RI.

Pipet tip quality
For the most accurate and precise pipetting results, the pipet manu-
facturer’s recommended tips should be used. Achieving a proper seal
between the pipet’s nose cone and the tip is critical for good perfor-
ance. Some generic tips may seemingly fit on a pipet, 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 pipets but still
introduced errors of up to ~0.6% RI and 0.8% CV, which would be addi-
tive to all other pipetting errors. If high-quality third-party tips are to be
used, it should be verified that they fit well and form a tight seal with the
intended pipet model.

Claimed pipet performance assumes the use of manufacturer’s tips.
When calibrating a pipet, it is imperative that it be calibrated with the
same tip type and under the same conditions of its use in the lab in order
to avoid errors when using the pipet for analytical tests.

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

Best pipetting practices
The results of this study demonstrate that even minor variation in the
operating technique of handheld air-displacement pipets can result
in measurable errors in accuracy and precision. This study did not
evaluate errors resulting from combining multiple of the discussed
technique variations, although this is commonly observed in the field.
Compounded errors can easily reach 12%, and are often even larger, as
data from field surveys suggest.

The following steps will ensure the most accurate and precise results:
• Prewet tips at least three times
• Use proper pipetting mode
• Work at temperature equilibrium
• Immerse tips to proper depth
• Aspirate with pipet in vertical position
• Pause after aspirating
• Do not touch vessel wall during or after aspiration
• Use consistent plunger speed and pressure
• Minimize heat transfer from hands
• Avoid tip wiping
• Examine tip prior to dispensing
• Use high-quality pipet tips
• Use proper pipet size.
References


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