Cool tips

0.28

0.34

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dead air volume, is trapped within the pipette as soon as the tip is immersed in the sample solution. Pipetting technique, as well as by choosing the appropriate pipette size and type of pipette tips.

pipettes (in this poster simply referred to as pipettes) render their performance susceptible to the captive air volume to the pipette's set volume will have on the actually aspirated and delivered volume of up to -1.1% and contribute up to 0.7% to the imprecision.

mode with aqueous solutions leads to significant over-delivery of up to 2.3% RI and contributes up to 1.5% to the error.

consistent with the pipette's manual delivery by a trend toward smaller delivered volumes, and lost -0.1% and 0.8% CV in this study.

Forward and Reverse Mode

Using the appropriate pipetting mode has one of the biggest influences on the accuracy of the volume delivery. Forward mode describes the pipetting technique in which the plunger of the pipette is depressed to the first stop, the pipette tip is immersed in the sample solution, and the sample then drawn by releasing the plunger. During delivery, the plunger is depressed beyond the first stop, to the so-called blow-out mode (second stop), forcing all the liquid out of the tip. Standard procedure for pipette calibration prescriptions 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. This leads to additional sample volume into the tip. The obtained volume is delivered by depressing the plunger to the first stop, retaining the additional sample in the tip. This pipetting mode is recommended for use with viscous or volatile solutions, however, using reverse mode with aqueous solutions leads to significant over-delivery of up to 2.2% CV and contributes up to 0.7% CV, as obvious from Figures 1 and 3.

The authors recommend specifying in the SOP for each tip, which pipette mode is to be used for the particular task(s) at hand. This will avoid errors induced by personal preference for one or the other pipetting mode.

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 tip's plunger, 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 under-delivery of up to -1.3% and contribute up to 0.7% to the imprecision.

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 tip 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 lead up to -0.9% CV and 0.18% CV in this study.

When dispensing the sample using forward mode, it is recommended to touch the tip-plunger to the side of the sample vessel, while the pipette tip 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 practise may lead to over-delivery of 0.5% CV over deliveries of 1.5 mm, and the tip depth 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.

Even small changes in the delivery tip may lead to an increased inaccuracy (up to 2.2% CV) of the delivered volumes.

Temperature Dis-equilibrium

For most accurate pipetting results, it is recommended that the pipette, tip-plunger tips, and the sample solution have been equilibrated for at least 2 hours and are within 0.5 °C of ambient temperature. Many samples 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[7]. The present study evaluated the use of tip-pipe tips, which had been cooled to 0 °C for 30 min prior to use. Pipetting with these tips led to significant under-delivery of sample of both pipette tips, contributing up to -2.9% and 2.7% to the error.

Heat Transfer / Hand Warming

Handing a pipette for prolonged periods of time will cause the barrel of the pipette to warm, leading to an expansion of the captive air volume. This heat transfer will ultimately impact the accuracy and precision, resulting in delivery of significantly smaller volumes than in the control experiment. The progressive warming of the barrel’s manual delivery by a trend toward smaller delivered volumes, and lost -1.9% and 0.8% CV in this study.

Influence of the Air Cushion on Pipette Performance

Pipette-aspirated air can be trapped within the pipette as soon as the tip is immersed in the sample solution. Pipetting technique, as well as by choosing the appropriate pipette size and type of pipette tips.

The ideal gas law allows to estimate the effect which temperature, evaporation, and the ratio of captive air to volume of the pipette's set volume will have on the actually aspirated and delivered volume of a pipetting cycle. The following theories studied here directly influence the captive air volume: Pre-wetting of Pipette Tips, Temperature Dis-equilibrium, Hand Warming, and Immersion Depth of Pipette Tip. Since the total volume of air can vary widely depending on the type of pipette, 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 pipette set at 20 μL, the other set with a 100 μL pipette set at 20 μL.

For best results, it is recommended to use variable-volume pipettes only to the nominal volume of the next available, smaller denomination of pipette. Several electronically operated pipettes offer an even wider range of selectable volumes.

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For the most accurate and precise pipetting results, the pipette manufacturer's tips should be used. Generic tips may seemingly fit on a pipette, but due to different taper angles of the various tip manufacturers’ the accuracy and precision, resulting in delivery of significantly smaller volumes than in the control experiment.

Avoid tip wiping

Avoid tip wiping

Minimize heat transfer from hands

Minimize heat transfer from hands

Use proper pipetting mode

Use proper pipetting mode

Work at temperature equilibrium

Work at temperature equilibrium

Immersion tip to proper depth

Immersion tip to proper depth

Aspirate with pipette in vertical position

Aspirate with pipette in vertical position

Pause after aspiration

Pause after aspiration

Do not touch vessel-well during and after aspiration

Do not touch vessel-well during and after aspiration

Use consistent plunger speed and pressure

Use consistent plunger speed and pressure

Avoid heat transfer from hands

Avoid heat transfer from hands

Avoid tip wiping

Avoid tip wiping

Examine tip prior to dispensing

Examine tip prior to dispensing

Use high quality tip-plunger tips

Use high quality tip-plunger tips

Use proper pipette size

Use proper pipette size

References