

Pipetting Hot and Cold

Observant scientists will have noticed that when they pipette cold samples they invariably aspirate a larger-than-expected sample volume each time the tip is changed. Oddly, a smaller volume is delivered from the second aspiration onward if the tip is not changed. The under-delivery may account for as much as 30 μ L when delivering 1000 μ L.

Since pipetting hot and cold samples is commonplace in the laboratory, this discrepancy is important. Numerous applications such as DNA extraction and amplification require cold liquid samples at 4°C, while the lab equipment remains at ambient temperature, usually around 20°C.

Pipetting hot samples while they are cooling after their incubation at 37°C produces the opposite effect, although there is no convenient way to verify when they actually reach room temperature.

These curious phenomena are attributable to the effect of temperature on air-displacement pipettes.

Electronic or manual air-displacement pipettes operate with a piston that moves inside a shaft. A certain level of air, called the air cushion, is maintained between the piston and liquid. Like any gas, the air cushion between the piston and a sample is defined by pressure, temperature, and volume. These variables are interrelated. Therefore the accuracy (systematic error) and precision (random error) of an air-displacement pipette correlate directly to the variation in the volume of the air cushion, which in turn varies with the temperature changes occurring inside the tips. Gilson conducted a study to determine the influence of sample temperature on pipette performance. Here we report the results and offer solutions to circumvent the issue. The work was performed in our ISO 17025-accredited laboratory, which is also accredited by the French Committee for Accreditation (COFRAC).

International Standard Incorporates Temperature Considerations

The International Organization for Standardization acknowledges that sample temperature affects pipetting functionality, for it states that calibration of pipettes should be conducted between 15 and 30°C, with a precision of +/- 0.5°C. Additionally, the instruments, consumables, and liquids used in the calibration process should be at room temperature, and their variation may entail accuracy errors of up to 0.3% per °C.

Testing Protocol and Observations

We developed a testing protocol to compare the sample volumes of consecutive pipetting cycles of distilled water at 4°C and 40°C with the standard reference of 20°C for calibrating pipettes. We performed 10 measurements of water, with the volume set at 100 μ L at both 4°C and at 40°C without changing tips in a 20°C regulated environment, using a Pipetman® P100 (volume range of 20 to 100 μ L) with Gilson Diamond® tips D200. When we aspirated a sample at 4°C for the first time, we observed that the dispensed volume was always higher than that obtained under standard conditions. Conversely, when we dispensed a sample at 40°C for the first time, the volume delivered was less than the reference.

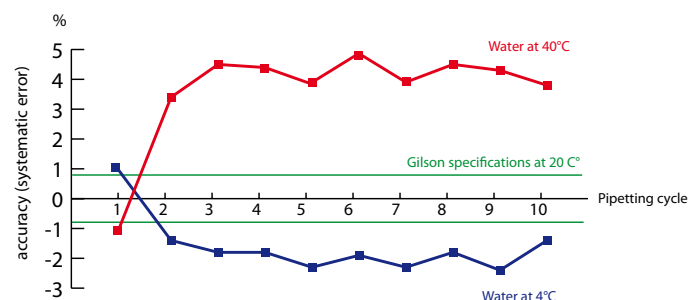


Figure 1. An evaluation of the accuracy of a Pipetman® P100 air displacement pipette as a function of sample temperature. Results were obtained from 10 measurements of water, with the volume set at 100 μ L at 4°C and at 40°C without changing tips, performed in Gilson's ISO 17025- and COFRAC-accredited laboratory.

We also observed a sharp inversion of this phenomenon in both cases as of the second pipetting cycle (Figure 1).

The systematic error ranges from four- to nine-fold above or below the volumetric specifications.

The first time water at 4°C is aspirated, the temperature of the air cushion that is in contact with the water drops, causing the air to shrink (Figure 2, cycle 1). Since the water and the air cushion share a fixed volume, the volume aspirated is higher than under standard conditions ($V_{\text{total}} = V_{\text{air cushion}} + V_{\text{sample}}$). During the second aspiration, the air cushion travels through a cold area limited by the tip walls that were previously in contact with the cold sample, to a tepid region corresponding to the tip holder area that is never in contact with the liquid (Figure 2, cycle 2). Consequently the air cushion temperature rises, causing the gas to expand. This corresponds to the exact opposite phenomenon we observed during the first cycle.

The conditions of the third and fourth pipetting cycle (Figure 2, cycle 3) are qualitatively similar to those of the second pipetting cycle, and a low volume also was reported.

The first time water at 40°C is aspirated, the temperature of the air cushion that is in contact with the water rises, causing the air to fill a greater volume. As a result, the sample volume dispensed is lower than what is expected under standard conditions. During the second aspiration, the air cushion travels from the warm area of the tip that was previously in contact with the water, to the tepid portion of the tip holder, which then causes a slight drop in the temperature and results in the air volume shrinking, producing a higher-than expected delivery volume.

From the third cycle onward, the delivery volume stabilizes below the standard value for cold samples and above for hot samples, but continues to fluctuate, albeit at a significantly lower rate. This demonstrates that reproducible results (random error) within the

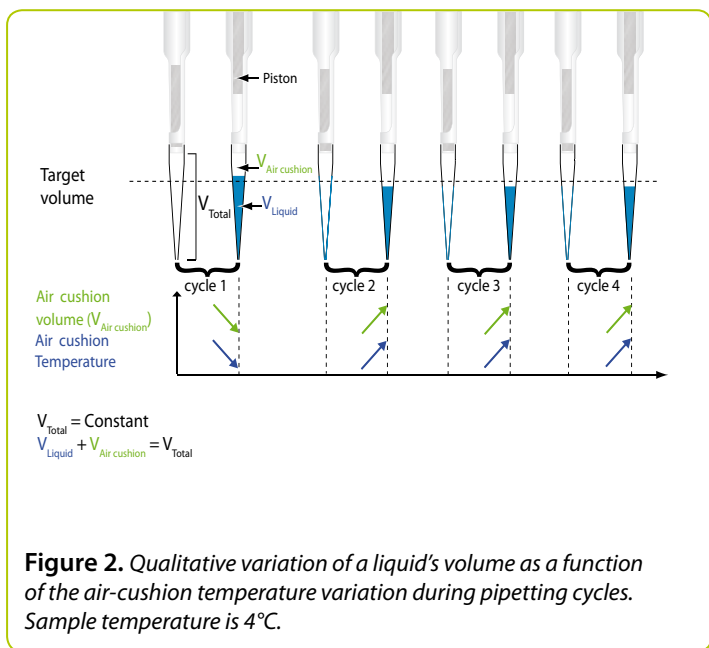


Figure 2. Qualitative variation of a liquid's volume as a function of the air-cushion temperature variation during pipetting cycles. Sample temperature is 4°C.

thresholds defined by the manufacturer's precision specifications cannot be obtained (Figure 1). Our findings indicate that accuracy (systematic error) drifts by as much as 0.1 to 0.2% per degree of difference between the sample temperature and 20°C. The more the temperature gap widens, the greater the gravimetric errors become, i.e., 4% for a sample at 40°C in an ambient temperature of 20°C.

Recommendations

We recommend that liquids and pipettes be allowed to equilibrate to ambient temperature. If that is not possible, researchers use certain techniques to offset the impact of temperature. One of them consists in changing tips without pre-wetting. As shown on figure 1 - cycle 1, the latter technique does not permit to obtain results within specifications. This collerates with the ISO 8655 standard that states that failure to prewet introduces up to 2% of error in measurement regardless of the sample temperature. Gilson does not recommend that technique but strongly recommends the pre-wetting technique which consists in aspirating and dispensing the sample liquid three or four times before aspirating for sample delivery. This technique, albeit inappropriate to enhance accuracy, permits to obtain a better reproducibility, a key factor in the most commonly used laboratory protocols involving repetitive pipetting tasks such as ELISA or PCR.

Researchers particularly concerned with data integrity can use positive displacement pipettes, which will enable them to work accurately and precisely above or below the ambient temperature and avoid possible drifting. The Gilson Microman® positive-displacement pipette uses a disposable capillary and piston, which isolate the aspirated sample from the inside part of the pipette, eliminating the air space between the piston and sample.

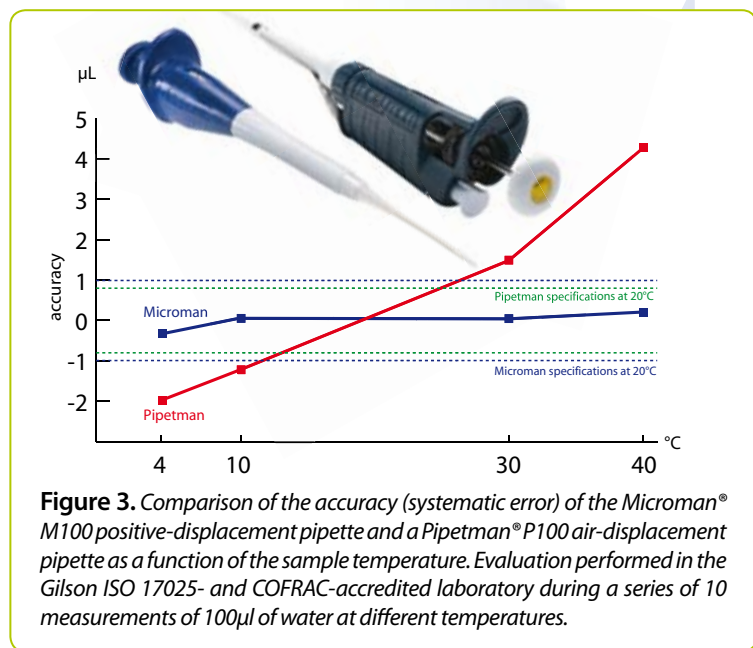


Figure 3. Comparison of the accuracy (systematic error) of the Microman® M100 positive-displacement pipette and a Pipetman® P100 air-displacement pipette as a function of the sample temperature. Evaluation performed in the Gilson ISO 17025- and COFRAC-accredited laboratory during a series of 10 measurements of 100µl of water at different temperatures.

The benefit of using the Microman® is that accuracy and precision remain consistent — whatever the sample temperature (Figure 3) — eliminating the use of specific pipetting techniques.

Biotechnology researchers, who handle genetic materials where inactivation of DNase and RNase is reached at 4°C, or laboratory diagnosticians working with biological liquids, such as serum at body temperature, cannot always wait for a liquid to reach the pipette temperature. Gilson recommends they pre-wet tips and apply the correction factor to ensure the most accurate and precise data. But the best alternative for obtaining excellent volumetric performances when unquestionable results are necessary at any sample temperature is to use positive-displacement pipettes like the Gilson Microman®.

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