Calibrating a Pipette

A pipette is used to precisely measure and transfer small volumes of liquid in the laboratory in experiments that demand high accuracy. However, the sources of error in pipette measurements could arise from device failure or due to misuse by the operator. Any discrepancy in volumes dispensed may affect the outcomes and reproducibility of an experiment such as qPCR results. It is, therefore, necessary to check pipette calibration every few months to ensure accuracy by dispensing right volumes. This is why pipette calibration is considered a fundamental part of good laboratory practice (GLP).

In a previous article, we discussed tips for preserving pipette accuracy — today we will learn how to perform pipette calibration.

Principles of Pipette Calibration

At 20°C temperature and one atmosphere of pressure, water density stays constant at 1 g/mL. The volume of water can be determined by weighing dispensed water. Temperature, atmospheric pressure, and humidity may influence the accuracy of measurements. These factors are combined to generate the Z factor, applied in the calculation of the volume of water, and compared with the theoretical volume, which finally determines the accuracy of the pipette.

Temperature (°C)	Z factor
20	1.0029
21	1.0031

22	1.0033
23	1.0035
24	1.0037
25	1.0039

Pipette calibration table of Z factors for distilled water at 1 atm pressure

Source: Wisconsin State Laboratory of Hygiene

Steps Involved in Pipette Calibration

- Take distilled water in a beaker and record its temperature. Also, gather your pipette and the correct tips based on both the small and large volumes that the pipette can dispense.
- Place a weigh boat on a balance that can accurately weigh in the microgram range, and set it to zero after closing the balance door.
- Pre-rinse the tip by aspirating and dispensing the set volume three times and push fully to remove any remaining liquid.
- Aspirate the calibration volume without bubble formation and dispense the liquid slowly into the weigh boat. Then, record the weight on the balance and repeat the process ten times.
- Calculate the dispensed volume by using the equation V = W x Z where W is the weight of the water, Z is the Z factor, and V is the calculated volume of dispensed water. Next, determine the mean value from ten trials.
- Finally, calculate accuracy by using the equation $A = 100 \times V_{avg}/V_0$, where A is the accuracy of the pipette, V_{avg} is the average calculated volume and V_0 is the theoretical volume you tried to dispense. If the accuracy value lies in the 99-101% range, the pipette is considered normal and calibrated.

Caution

Large changes in the testing environment, such as temperature, humidity, and atmospheric pressure, may impact evaporation during calibration, which could eventually lead to an unreliable test. It is also important to adjust the pipettes to the calibration environment for more than an hour before starting the calibration. Lastly, if the pipette does not pass the calibration test and you cannot fix the calibration yourself, then, have it set up for service.