
Pipette calibration

1. Principle:

Under a constant temperature and atmospheric pressure, the density of distilled water is constant. The volume of water can be determined by weighting dispensed water. The calibration of pipette is carried out by gravimetric method. When determining the volume of water, the accuracy of measurements is effected by ambient temperature, atmospheric pressure and relative humidity. These factors are usually combined to give the Z factor, used in calculation of volume of water. Then the calculated volume of water is compared with the theoretical volume to determine the accuracy and precision of the pipette.

2. Material and equipment:

- (1) pipette and tips
- (2) 50 ml beaker and plastic medicine cup
- (3) distilled water ($\geq 10\text{MQ}$)
- (4) temperature meter ($\pm 0.1^\circ\text{C}$)
- (5) Analytical balance ($\pm 1.0\text{ mg}$)
- (6) Atmospheric pressure meter

3. Procedure

- (1) Determine the water temperature and record it.
- (2) Place a beaker filled with distilled water into analytic balance and close the door of balance waiting for equilibrium of inner vapor
- (3) Place a plastic medicine cup on the pan and adjust the weight to zero.
- (4) Put a tip onto the pipette and set the volume which is to be tested.
- (5) Pre-rinse the tip: aspirate and dispense the setting volume three times and press the push – button on the second stop to remove any remaining liquid.
- (6) Press the push-button to the first positive stop. Hold the pipette vertically, immerse the tip so that 1-4 mm in the liquid and release the push button slowly and smoothly to aspirate the liquid.
- (7) Wait one second and withdraw the tip from the liquid.
- (8) Wipe any droplets away from the outside of the tip using a kimwipe.
- (9) Place the end of the tip against the inside wall of the plastic cup at an angle of $10-40^\circ$.
- (10) Press the push-button smoothly to the first stop. Wait one second, change new site and press the push-button on the second stop.
- (11) Keeping the push-button press to the end, remove the pipette by drawing the tip along the inside

surface of the plastic cup and release the push button.

- (12) Close the door of balance and record the value on the balance display after it has stabilized.
- (13) Repeat step (6) to (12) 14 times (the weight display of balance should be adjusted to zero every time)
- (14) Eject the tip.
- (15) Pipette should be calibrated every three months routinely.

4. Calculation:

- (1) Convert the weight unit of measured value into the volume unit of measured value using the following formula:

$$\text{Volume (ml or ul)} = \text{Weight (mg or ug)} \times Z$$

Z value : conversion factor, which is conversion of density (see table 1)

- (2) Calculate the average (Mean), accuracy, standard deviation (S.D.) and imprecision (C.V.) using the following formula:

$$(\text{Mean}) X = \frac{\sum_{i=1}^n X_i}{N} ; \quad \text{S.D.} = \left(\frac{\sum_{i=1}^n (X_i - X)^2}{N} \right)^{1/2}$$

N: number of measured value

$$\text{C.V.} = \frac{\text{S.D.}}{\text{Mean}} \times 100\% ; \quad \text{Accuracy} = \frac{\text{Measured value}}{\text{Theoretical value}} \times 100\%$$

- (3) Accuracy value must be 99-101 % and C.V. value must be less than 1 %.

Pipette Calibration Record

Pipette number : _____ ; Volume of pipette : _____

Water temperature : _____ ; Atmospheric pressure : _____ ; Z value : _____

Operator : _____ ; Date : _____

Weight record (mg):

1		6		11	
2		7		12	
3		8		13	
4		9		14	
5		10		15	

Formula :

$$(\text{Mean}) \bar{X} = \frac{\sum_{i=1}^n X_i}{N} ; \quad \text{S.D.} = \left(\frac{\sum_{i=1}^n (X_i - \bar{X})^2}{N} \right)^{1/2}$$

$$\text{C.V.} = \frac{\text{S.D.}}{\text{Mean}} \times 100\% ; \quad \text{Accuracy} = \frac{\text{Measured value}}{\text{Theoretical value}} \times 100\%$$

Record :

Mean= _____ ; Accuracy= _____ ; C.V.= _____

Table 1. Values for Z (ug/mg), as a function of temperature and atmospheric pressure, for distill water

	800 mbar	853 mbar	907 mbar	960 mbar	1013 mbar	1067 mbar
15.0°C	1.0018	1.0018	1.0019	1.0019	1.002	1.002
15.5°C	1.0018	1.0019	1.0019	1.002	1.002	1.0021
16.0°C	1.0019	1.002	1.002	1.0021	1.0021	1.0022
16.5°C	1.002	1.002	1.0021	1.0022	1.0022	1.0023
17.0°C	1.0021	1.0021	1.0022	1.0022	1.0023	1.0023
17.5°C	1.0022	1.0022	1.0023	1.0023	1.0024	1.0024
18.0°C	1.0022	1.0023	1.0024	1.0024	1.0025	1.0025
18.5°C	1.0023	1.0024	1.0025	1.0025	1.0026	1.0026
19.0°C	1.0024	1.0025	1.0025	1.0026	1.0027	1.0027
19.5°C	1.0025	1.0026	1.0026	1.0027	1.0028	1.0028
20.0°C	1.0026	1.0027	1.0027	1.0028	1.0029	1.0029
20.5°C	1.0027	1.0028	1.0028	1.0029	1.003	1.003
21.0°C	1.0028	1.0029	1.003	1.003	1.0031	1.0031
21.5°C	1.003	1.003	1.0031	1.0031	1.0032	1.0032
22.0°C	1.0031	1.0031	1.0032	1.0032	1.0033	1.0033
22.5°C	1.0032	1.0032	1.0033	1.0033	1.0034	1.0035
23.0°C	1.0033	1.0033	1.0034	1.0035	1.0035	1.0036
23.5°C	1.0034	1.0035	1.0035	1.0036	1.0036	1.0037
24.0°C	1.0035	1.0036	1.0036	1.0037	1.0038	1.0038
24.5°C	1.0037	1.0037	1.0038	1.0038	1.0039	1.0039
25.0°C	1.0038	1.0038	1.0039	1.0039	1.004	1.0041
25.5°C	1.0039	1.004	1.004	1.0041	1.0041	1.0042
26.0°C	1.004	1.0041	1.0042	1.0042	1.0043	1.0043
26.5°C	1.0042	1.0042	1.0043	1.0043	1.0044	1.0045
27.0°C	1.0043	1.0044	1.0044	1.0045	1.0045	1.0046
27.5°C	1.0044	1.0045	1.0046	1.0046	1.0047	1.0047
28.0°C	1.0046	1.0046	1.0047	1.0048	1.0048	1.0049
28.5°C	1.0047	1.0048	1.0048	1.0049	1.005	1.005
29.0°C	1.0049	1.0049	1.005	1.005	1.0051	1.0052
29.5°C	1.005	1.0051	1.0051	1.0052	1.0052	1.0053
30.0°C	1.0052	1.0052	1.0053	1.0053	1.0054	1.0055