

ali-Q™ Gravimetric Verification Procedure

Introduction

This document describes the recommended procedure for verifying performance of the ali-Q® aliquoting pipet controller using the industry standard- **gravimetric method**. ISO 8655 states that the primary methodology for measurement of motorized pipet controllers is through gravimetric measurement analysis with appropriate equipment under controlled environment. This is the most accurate and reliable way of determining whether your ali-Q delivers accurate and precise aliquot volumes that are within the manufacturers published performance specifications.

If access to the specified equipment is not available, VistaLab Technologies, Inc. maintains a qualified service and repair center which can perform repair service and calibration verification. Contact VistaLab Technical Services for more information.

Materials:

Balance	<ul style="list-style-type: none">•Balance should be capable of weighing to a minimum of 3 decimal places (0.001g).•Balance should be regularly serviced and certified by a qualified technician, using weights traceable to the National Institute of Standards and Technology (NIST). Between service calls, balance should be qualified using NIST traceable weights; and they should be confirmed for stability, integration time, and levels.•Balance should be stationed on marble tables or balance tables mounted on elastomeric vibration isolator pads to minimize vibration.•Balance environment should be humidified to prevent evaporation of the dispensed test volume.•Balances should be turned on at least one hour prior to use.
Weighing Vessel	Weighing vessels should be narrow mouthed and their volume capacity should be a minimum of 10x the test volume. In an especially arid climate, vessels with covers to minimize evaporation are suggested
Thermometers	Thermometers should be calibrated and readable to 0.1°C to measure the temperature of the room and temperature of the water.
Hygrometer	Hygrometer should be calibrated and readable to the nearest whole percentage to measure the relative humidity of the environment. Otherwise, access the internet and search for your local relative humidity.
Barometer	Barometer should be calibrated and readable to 0.1°mmHg to measure the atmospheric pressure of the environment. Otherwise, access the internet and search for your local atmospheric pressure.
Serological Pipets	ali-Q is compatible with all makes of serological pipets, but they are factory calibrated for optimal performance when used with Wobble-not serological pipets.
Water	Non-aerated deionized or distilled water that has been allowed to equilibrate to room temperature in an appropriate container for at least two (2) hours prior to testing..

Environment

A controlled environment is recommended to ensure test reliability. Fluctuations in room temperature and humidity will adversely affect data. Maintain the following laboratory conditions for at least two (2) hours prior to, and throughout, the verification procedure. Ensure that balances and pipets are properly equilibrated to ambient conditions by allowing them to equilibrate to the environment at least two (2) hours prior to verification.

Temperature	Air: $21.5 \pm 1.0^{\circ}\text{C}$ measured to 0.1°C Water: $21.5 \pm 1.0^{\circ}\text{C}$ measured to 0.1°C
Relative Humidity	45-75% RH measured to 1.0%
Barometric Pressure	measured to $\pm 20\text{mmHg}$, 25mbar, 0.15kpa, or 0.7inHg
Other Conditions	Drafts should be minimized.

When to Verify

All ali-Q pipet controllers manufactured and serviced by VistaLab Technologies, Inc. are shipped with a calibration certificate that is traceable to NIST.

Some laboratory-specific procedures call for an internally performed verification prior to placing any new pipetting device into routine use. It is recommended that ali-Q pipet controllers be verified for accuracy and precision whenever any of the following conditions occur:

1. Routinely, every 6 - 12 months
2. If quality control or experimental results are unacceptable
3. If any maintenance, other than cleaning of the outer surfaces or changing the nozzle filter has been performed.
4. As required by your labs' internal governing regulatory body (ISO, CLIA, CAP, etc)

ali-Q Operation

1. Attach serological pipet
2. Aspirate as much water needed to perform 10 measurements of desired verification volume
3. Set the purple aliquot volume-set dial to the desired aliquot volume
4. Aliquot - Carefully position the tip of the full pipet over the weighing vessel. Press and HOLD the purple aliquot button and the LED will turn solid GREEN. Hold button until green LED turns off and motor turns on. Repeat for each aliquot.
NOTE: If you release the aliquot button too soon, i.e. before the full aliquot volume has dispensed, the LED will blink RED and you will hear an "alert" sound. The aliquot did not dispense fully/properly, and will not be accurate. Re-do the aliquot.
NOTE: It is recommended to discard (tare) the first aliquot for optimal precision



Verification Procedure

Gravimetric verification procedure outlined in this document is compliant and consistent with methodologies outlined ISO 8655.

Environmental conditions should be controlled and recorded at the time of testing. Measuring equipment should be regularly calibrated and verified and appropriate for the volume being measured. Based on industry guidelines and recommendations, any pipet that is tested by other than the original instrument manufacturer should be tested using at least ten (10) data points for performance verification.

1. Record all ambient conditions read from thermometer, barometer and hygrometer. If measurement equipment unavailable, do an internet search for the current local conditions.
2. Determine your Z-factor using the chart in Appendix A and the values recorded from water thermometer and barometer.
3. Measure and record at least 10 aliquot dispenses on your balance at each volume of interest. VistaLab performs verification at 0.5mL and 5.0mL only. Customers should at least verify at both 0.5mL and 5.0mL, but can verify at any volume between.
4. Calculate the Accuracy (%) and Precision (CV%) for all the data points measured using the equations below.
 - a. Calculate the Mean Mass (M_{avg}) according to Equation 1
 - b. Calculate the Mean Volume (V_{avg}) according to Equation 2
 - c. Calculate the Accuracy (%) according to Equation 3
 - d. Calculate the Precision (CV%) according to Equations 4 and 5
5. Compare your results from Step 3 to the published manufacturers performance specs listed below to deem performance acceptable or not
6. Adjust the aliquot volume delivery by either:
 - a. Slightly turning the dial up or down based on your measurements.
 - b. Using the ali-Q Customer Verification Software
7. Repeat verification after any adjustments

Equations:

M_i = Mass of indiv. weight measurement (g)
 n = # of measurements (in this case, 10)

1. Mean Mass (M_{avg}) =

$$\frac{M_i}{n}$$

2. Mean Volume (V_{avg})=

$$(M_{avg}) \times (Z\text{-Factor})$$

3. Accuracy (%) =

$$\frac{(V_{avg} - V_{target})}{(V_{target})} \times 100$$

4. Standard Deviation (SD)

$$SD = \sqrt{\frac{\sum M_i^2 - \frac{(\sum M_i)^2}{n}}{n - 1}}$$

5. Precision (CV%)

$$CV\% = \frac{SD}{M_{avg}} \times 100$$

APPENDIX A

Z Factor Chart (mL/g) for water

To find the Z factor, locate the water temperature closest to the temperature measured during the test, then follow along that row to the column that represents the nearest Barometric Pressure measured during the test. That number is the Z factor (e.g., 18.0°C and 680 mm Hg = 1.0024 Z factor).

Barometric Pressure

mm Hg	600	640	680	720	760	800
mbar	800	853	907	960	1013	1067
kPa	80.0	85.3	90.7	96.0	101.3	106.7
in. Hg	23.6	25.2	26.8	28.3	29.9	31.5

Water Temperature °C

15.0	1.0018	1.0018	1.0019	1.0019	1.0020	1.0020
15.5	1.0018	1.0019	1.0019	1.0020	1.0020	1.0021
16.0	1.0019	1.0020	1.0020	1.0021	1.0021	1.0022
16.5	1.0020	1.0020	1.0021	1.0022	1.0022	1.0023
17.0	1.0021	1.0021	1.0022	1.0022	1.0023	1.0023
17.5	1.0022	1.0022	1.0023	1.0023	1.0024	1.0024
18.0	1.0022	1.0023	1.0024	1.0024	1.0025	1.0025
18.5	1.0023	1.0024	1.0025	1.0025	1.0026	1.0026
19.0	1.0024	1.0025	1.0025	1.0026	1.0027	1.0027
19.5	1.0025	1.0026	1.0026	1.0027	1.0028	1.0028
20.0	1.0026	1.0027	1.0027	1.0028	1.0029	1.0029
20.5	1.0027	1.0028	1.0028	1.0029	1.0030	1.0030
21.0	1.0028	1.0029	1.0030	1.0030	1.0031	1.0031
21.5	1.0030	1.0030	1.0031	1.0031	1.0032	1.0032
22.0	1.0031	1.0031	1.0032	1.0032	1.0033	1.0033
22.5	1.0032	1.0032	1.0033	1.0033	1.0034	1.0035
23.0	1.0033	1.0033	1.0034	1.0035	1.0035	1.0036
23.5	1.0034	1.0035	1.0035	1.0036	1.0036	1.0037
24.0	1.0035	1.0036	1.0036	1.0037	1.0038	1.0038
24.5	1.0037	1.0037	1.0038	1.0038	1.0039	1.0039
25.0	1.0038	1.0038	1.0039	1.0039	1.0040	1.0041
25.5	1.0039	1.0040	1.0040	1.0041	1.0041	1.0042
26.0	1.0040	1.0041	1.0042	1.0042	1.0043	1.0043
26.5	1.0042	1.0042	1.0043	1.0043	1.0044	1.0045
27.0	1.0043	1.0044	1.0044	1.0045	1.0045	1.0046
27.5	1.0044	1.0045	1.0046	1.0046	1.0047	1.0047
28.0	1.0046	1.0046	1.0047	1.0048	1.0048	1.0049
28.5	1.0047	1.0048	1.0048	1.0049	1.0050	1.0050
29.0	1.0049	1.0049	1.0050	1.0050	1.0051	1.0052
29.5	1.0050	1.0051	1.0051	1.0052	1.0052	1.0053
30.0	1.0052	1.0052	1.0053	1.0053	1.0054	1.0055

Pipette Accuracy and Precision Verification Worksheet

Test Conditions

Serial# _____
 Target Volume _____ mL
 Air Temperature _____ °C
 Water Temperature _____ °C
 Humidity _____ %
 Barometric Pressure _____
 Balance Serial No. _____
 Balance Model _____
 Z-factor (from Appendix A) _____

Weight Measurements

Mass	Mass ²
M ₁ _____ g	M ₁ ² _____ g ²
M ₂ _____ g	M ₂ ² _____ g ²
M ₃ _____ g	M ₃ ² _____ g ²
M ₄ _____ g	M ₄ ² _____ g ²
M ₅ _____ g	M ₅ ² _____ g ²
M ₆ _____ g	M ₆ ² _____ g ²
M ₇ _____ g	M ₇ ² _____ g ²
M ₈ _____ g	M ₈ ² _____ g ²
M ₉ _____ g	M ₉ ² _____ g ²
M ₁₀ _____ g	M ₁₀ ² _____ g ²
ΣM _i _____ g	ΣM _i ² _____ g ²
(M _{avg}) _____ g	

Accuracy (%)

$$V_{avg} = (M_{avg}) \times (Z\text{-Factor}) =$$

$$\text{_____ mL} = (\text{_____}) \times (\text{_____})$$

$$\text{Accuracy (\%)} = [(V_{avg} - V_{target}) / (V_{target})] \times 100$$

$$\text{_____ \%} = [(\text{_____} - \text{_____}) / \text{_____}] \times 100$$

Standard Deviation (SD)

$$SD = \sqrt{\frac{\sum M_i^2 - \frac{(\sum M_i)^2}{n}}{n - 1}}$$

$$SD = \sqrt{\frac{(\text{_____}) - (\text{_____})}{\text{_____}}}$$

$$= \text{_____}$$

Precision (CV%)

$$CV\% = \frac{SD}{(M_{avg} + e_{avg})} \times 100$$

$$= [(\text{_____} / \text{_____} + \text{_____})] \times 100$$

$$= \text{_____}$$

Performed by: _____

Date: _____

* 1000µL = 1 mL. To convert microliters to milliliters, divide microliters by 1000.

SAMPLE

Pipette Accuracy and Precision Verification Worksheet

Test Conditions

Serial# _____
 Expected Volume 1.0 mL
 Air Temperature 23.0 °C
 Water Temperature 22.0 °C
 Humidity 63.0 %
 Barometric Pressure 29.4
 Balance Serial No. _____
 Model _____
 Z factor (from Appendix A) 1.0033

Weight Measurements

Mass	Mass ²
M ₁ <u>0.98501</u> g	M ₁ ² <u>0.970245</u> g ²
M ₂ <u>0.98895</u> g	M ₂ ² <u>0.978022</u> g ²
M ₃ <u>0.98766</u> g	M ₃ ² <u>0.975472</u> g ²
M ₄ <u>0.98660</u> g	M ₄ ² <u>0.973380</u> g ²
M ₅ <u>0.98522</u> g	M ₅ ² <u>0.970658</u> g ²
M ₆ <u>0.98523</u> g	M ₆ ² <u>0.970678</u> g ²
M ₇ <u>0.98700</u> g	M ₇ ² <u>0.974169</u> g ²
M ₈ <u>0.98627</u> g	M ₈ ² <u>0.972729</u> g ²
M ₉ <u>0.98420</u> g	M ₉ ² <u>0.968650</u> g ²
M ₁₀ <u>0.986305</u> g	M ₁₀ ² <u>0.973991</u> g ²
ΣM _i <u>9.86305</u> g	ΣM _i ² <u>9.727994</u> g ²
M _{avg} <u>0.986305</u> g	

Accuracy (%)

$$V_{\text{avg}} = (M_{\text{avg}}) \times (\text{Z-Factor}) =$$

$$\underline{\hspace{2cm}} \text{ mL} = (\underline{\hspace{1cm}}) \times (\underline{\hspace{1cm}})$$

$$\text{Accuracy (\%)} = [(V_{\text{avg}} - V_{\text{target}}) / (V_{\text{target}})] \times 100$$

$$\underline{\hspace{2cm}} \% = [(\underline{\hspace{1cm}} - \underline{\hspace{1cm}}) / \underline{\hspace{1cm}}] \times 100$$

Standard Deviation (SD)

$$SD = \sqrt{\frac{\sum M_i^2 - \frac{(\sum M_i)^2}{n}}{n - 1}}$$

$$SD = \sqrt{\frac{9.727994 - 9.727976}{9}}$$

$$= \underline{\underline{0.0014142}}$$

Precision (CV%)

$$CV\% = \frac{SD}{(M_{\text{avg}} + e_{\text{avg}})} \times 100$$

$$= [\underline{0.0014} / \underline{0.986305} + \underline{0.0030}] \times 100$$

$$= \underline{\underline{0.14}}$$

Performed by: _____

Date: _____

* 1000µL = 1 mL. To convert microliters to milliliters, divide microliters by 1000.