



ACCREDITATION UNIT

CALIBRATION OF PISTON PIPETTES USING GRAVIMETRIC METHOD

Purpose:

This Policy is designed to help laboratories with the internal calibration of Piston Pipettes using Gravimetric Method.

Scope:

This Policy is applicable for the internal calibration of the piston-operated volumetric pipettes that are used to transport a measured volume of liquid within laboratories.

Authorship:

This Publication has been written by the Technical Committee, and approved by the Accreditation Unit Director

Official Language:

The text may be translated into other language as required. The English language version remains the definitive version.

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Further Information:

It is available at AU web site: www.au.gov.jo, where check updates can be checked directly.

Contact us:

Accreditation Unit (AU)

P.O. box 941287 Amman - 11194 Jordan

Tel: +962 6 5658036

Fax: +962 6 5695721

E-mail: Imarashdeh@jsmo.gov.jo

Contents:

Subject	Page
1. Introduction	3
3. Responsibilities	3
4. Policy	3
5. References	7

1. Introduction:

This Publication is Policy use and checking of piston pipettes by scientists, technicians and other laboratory workers.

2. Responsibilities:

- a. It is the responsibility of AU assessors to evaluate the compliance of the laboratories with this Policy.
- b. Laboratories heads and supervisors are responsible for enforcement and compliance with the safety policy in the laboratory.
- c. Employees of the laboratories are responsible to implement the safety guideline requirements.

3. Policy

3.1 Apparatus:

- a. Analytical balance, with a resolution appropriate to the selected volume of the apparatus under test according to Table (1) below.

The nominal volume of apparatus under test V (µl)	Resolution (mg)	Repeatability and Linearity (mg)	Standard uncertainty of measurement (mg)
$1 \leq V \leq 10$	0.001	0.002	0.002
$10 < V \leq 100$	0.01	0.02	0.02
$100 < V \leq 1000$	0.1	0.2	0.2
$1\ 000 < V \leq 10\ 000$	0.1	0.2	0.2
$10\ 000 < V \leq 200\ 000$	1	2	2

- b. Liquid reservoir, with sufficient capacity for all the test liquid likely to be required for the complete series of tests.
- c. Weighing vessel, suitable for the test procedure, it is preferred to have a lid to eliminate the loss of water by evaporation during the dispensing and weighing procedure.
- d. Thermometer, with a standard uncertainty of $\leq \pm 0.2$ oC.
- e. Hygrometer, with a standard uncertainty of $\leq \pm 10\%$.
- f. Test liquid, use distilled or deionized water conforming grade 3 as specified in ISO 3696, degassed or air-equilibrated. The water shall be at room temperature.

3.2 Test Conditions:

- a. The relative humidity $\geq 50\%$; and
- b. Temperature between 15 °C and 30 °C, constant within (± 0.5 °C).

Note: Prior to calibration, the pipettes, test water and apparatus should be left at room temperature for 2 hours at least.

3.3 Test Volume:

- a- In the case of a variable-volume (user-selectable volume) pipettes, test three volumes at least as follows:
 - i. The nominal volume,
 - ii. Approximately 50% of the nominal volume,
 - iii. The lower limit of the useful volume range or 10% of the nominal volume (whichever is the greater).
- b- In case of fixed volume, test the nominal volume.

3.4 Procedure:

3.4.1 Record the Ambient temperature, relative humidity, and water temperature in form no. **qf-JAS-P17-001**

3.4.2 Fit the selected tip to the piston pipette.

3.4.3 Fill the tip with test liquid and expel to waste five times to reach humidity equilibrium in the dead air volume.

3.4.4 Place the weighing vessel with its added water onto the balance pan.

3.4.5 Tare the balance to zero with the vessel on it.

3.4.6 Replace the disposable tip of the piston pipette.

3.4.7 Fill the piston pipette with test liquid, immersing its delivery orifice 2 mm to 3 mm below the surface of the water. Release the operating button slowly, if hand operated, and withdraw the pipette vertically and carefully from the surface of the water. Touch the delivery orifice against the side wall of the container with the test liquid.

3.4.8 Expel the water to waste in order to pre-wet the tip and refill the piston pipette as described in (3.4.7).

3.4.9 If the weighing vessel has a lid, remove it. Deliver the contents of the pipette into the weighing vessel, touching the delivery end of the pipette tip against the inside wall of the vessel just above the liquid surface at an angle of approximately 30° to 45° and draw it approximately 8 mm to 10 mm along the inner wall of the weighing vessel to

remove any droplets at or around the tip orifice (remaining Liquid). Replace the lid if applicable.

3.4.10 Return the weighing vessel to the balance pan after delivery,

3.4.11 Record the mass of expelled water in the weighing vessel (m1) on form no **qf-JAS-P17-001** then tare the balance to zero with the vessel on it,

3.4.12 Repeat the test cycle described in points from (3.4.7 to point 3.4.11) above 10 times and record the reading every time.

3.4.13 After the last weighing leave the weighing vessel on the balance pan for 30 s and record its mass (me). If a weighing vessel with lid is used, omit this step.

Notes:

-In the case of variable volume pipettes take 10 readings for every test volume.

-Don't change the selected volume until the end of the 10 readings.

3.5 Calculations:

Use the Excel sheet "calibration program of piston pipettes **JAS-P17-Part I**" to calculate the average volume, systematic error, random errors, and uncertainty.

Expelled Volume (V) = mass of expelled water (m1)* Z correction factor

▼ Systematic error:

$$\text{Systematic Error } (\mu\text{g}) = \text{mean value} - \text{measured value} \quad \dots \dots \dots (1)$$

$$\text{Systematic Error } (\%) = 100 * (\text{mean value} - \text{measured value}) \quad \dots \dots \dots (2)$$

Random error:

Standard deviation of the readings in repeatability:

$$S_r = \sqrt{\frac{\sum_{i=1}^n (V_i - \bar{V})^2}{n-1}} \quad \dots \dots \dots (3)$$

The coefficient of variation, CV:

$$\text{CV}(\%) = \left(\frac{S_r}{\bar{V}}\right) * 100 \quad \dots \dots \dots (4)$$

Where;

Sr: repeatability

Vi: individual volume (calculated)

\bar{V} : Mean of the individual volumes from the series

n: Number of weighing in the series

CV: variation coefficient

Table no. (2) The Z correction factors for distilled water as a function of test temperature and

air pressure.

Z values in microliters per milligram

Temperature, °C	Air pressure	
	95 kPa	100 kPa
15.0	1.0019	1.0020
15.5	1.0020	1.0020
16.0	1.0021	1.0021
16.5	1.0021	1.0022
17.0	1.0022	1.0023
17.5	1.0023	1.0024
18.0	1.0024	1.0025
18.5	1.0025	1.0025
19.0	1.0026	1.0026
19.5	1.0027	1.0027
20.0	1.0028	1.0028
20.5	1.0029	1.0029
21.0	1.0030	1.0031
21.5	1.0031	1.0032
22.0	1.0032	1.0033
22.5	1.0033	1.0034
23.0	1.0034	1.0035
23.5	1.0036	1.0036
24.0	1.0037	1.0037
24.5	1.0038	1.0039
25.0	1.0039	1.0040
25.5	1.0041	1.0041
26.0	1.0042	1.0042
26.5	1.0043	1.0044
27.0	1.0045	1.0045
27.5	1.0046	1.0047
28.0	1.0047	1.0048
28.5	1.0049	1.0049
29.0	1.0050	1.0051
29.5	1.0052	1.0052
30.0	1.0053	1.0054

Table no. (3) Maximum permissible error (MPE).

Nominal Volume μl	Maximum Permissible systematic error		Maximum Permissible random error	
	$\pm \%$	$\pm \mu\text{l}$	$\pm \%$	$\pm \mu\text{l}$
1	5,0	0,05	5,0	0,05
2	4,0	0,08	2,0	0,04
5	2,5	0,125	1,5	0,075
10	1,2	0,12	0,8	0,08
20	1,0	0,2	0,5	0,1
50	1,0	0,5	0,4	0,2
100	0,8	0,8	0,3	0,3
200	0,8	1,6	0,3	0,6
500	0,8	4,0	0,3	1,5
1 000	0,8	8,0	0,3	3,0
2 000	0,8	16	0,3	6,0
5 000	0,8	40	0,3	15,0
10 000	0,6	60	0,3	30,0

Notes:

- 1- Pipettes with a nominal volume not mentioned in table (3), take the nearest higher volume for it.
- 2- For multichannel pipettes the MPE equal twice the values mentioned in table no. (3).
- 3- For adjustable volume pipettes the MPE equal the values mentioned in table no. (3) For the nominal volume.

4. References

1. Piston-operated volumetric apparatus -- Part 2: Piston pipettes ISO 8655-2:2002
2. Piston-operated volumetric apparatus -- Part 6: Gravimetric methods for the determination of measurement error ISO 8655-6:2002.

5. Related Documents

The following documents are defined as quality records for the purpose of Section 5.4 of ISO/IEC 17011:

- Calibration program of piston pipettes-**JAS-P17**-Part I.
- Weighing results - **qf-JAS-P17-001**
- Uncertainty calculation (**JAS-P17**-Part II)