Standard Operating Procedure

Calibration and Operation of Pipettes

Modified from ISO 8655-2:2002 and ASTM E1154-14:2014

1. Introduction

1.1. Purpose

To outline the procedure for operation and calibration of pipettes in the TRACES Centre. This procedure describes how to calibrate a volumetric air displacement pipette gravimetrically using the addition-tare method.

Best practices of pipette operation are described in detail.

1.2. Scope

Applicable to TRACES Centre pipetting equipment.

1.3. Responsibility

TRACES Staff

1.4. Accountability

TRACES Manager

2. Referenced Documents

2.1. ISO 8655-1:2002, Piston-operated volumetric apparatus — Part 1: Terminology, general requirements and user recommendations
2.2. ISO 8655-2:2002, Piston-operated volumetric apparatus — Part 2: Piston Pipettes
2.3. ISO 8655-6:2002, Piston-operated volumetric apparatus — Part 6: Gravimetric methods for the determination of measurement error
2.4. ASTM E1154-14:2014, Standard Specification for Piston or Plunger Operated Volumetric Apparatus
2.5. ASTM E542-01:2012, Standard Practice for Calibration of Laboratory Volumetric Apparatus
2.6. ASTM D1193-91:ASTM Standards for Laboratory Reagent Water
2.7. ISO17025:2017, General requirements for the competence of testing and calibration laboratories

3. Equipment

3.1. **Analytical balance**, with a resolution appropriate to the selected volume of the apparatus under test (see table A.1, appendix A)
3.2. **Liquid reservoir,** appropriately sized beaker rinsed 3 times with type 1 deionized water
3.3. **Weighing vessel**, 10 mL, 25 mL, or 250 mL Erlenmeyer flask rinsed 3 times with type 1 deionized water
3.4. **Timing device,** with a standard uncertainty of ≤ 1 s (k=1)

3.5. Thermometer, with a standard uncertainty of ≤ 0.2 °C (k=1)
3.6. Hygrometer, with a standard uncertainty of ≤ 10 % (k=1)
3.7. Barometer, with a standard uncertainty of ≤ 0.5 kPa (k=1)
3.8. Room temperature distilled or deionized water, conforming Type 1, as specified in ISO 3696, degassed or air-equilibrated

4. Test Conditions and Metrological Performance Requirements

4.1. Calibration of variable volume pipettes in the TRACES Centre must be completed every 3-months.

4.2. Each pipette shall be operated as specified in the supplier's operation manual. Refer to each individual supplier manual as recommended operating conditions may vary.

Note: All supplier manuals are in the original pipette boxes which can be found above the pipette storage cabinet in the TRACES Centre.

4.3. All pipettes being tested as well as the test water must sit in the test room for a minimum of 2 h before testing begins.

4.4. Tests should be completed in a draught-free and stable environment.

4.4.1. Humidity: relative humidity must be above 50 %

4.4.2. Temperature: constant (± 0.5 °C) between 15-30 °C

4.5. The time required to complete the weighing of one dispensed volume must be kept to a minimum and should ideally not exceed 60 s.

4.5.1. It is important the cycle time is consistent within a given cycle as well as from cycle to cycle.

5. Procedures

5.1. Pipette Operation

For all purposes of use in the TRACES Centre pipettes will be operated in the forward mode.

5.1.1. Aspirating Liquid:

a) Attach pipette tip firmly, ensure good contact by gently pushing up on the tip.
b) Press the push-button of the pipette down to the intermediate stop position.
c) Immerse the pipette tip vertically into the water.

Table 1. Appropriate immersion depth based on working variable pipette volume range

Volume, µL	Immersion Depth, mm
1 to 100	2 to 3
101 to 1000	2 to 4
1.1 to 10 mL	3 to 6
Reproduced from ASTM E1154-14:2014	

d) Allow the push-button to move up to the top stop position slowly and smoothly.

5.1.2. Dispensing Liquid:

a) Place the pipette tip at an angle between 10-45° against the inside wall of the receiving vessel.
b) Slowly press the push-button down to the first stop to empty the tip and wait 1 s.
c) To ensure the tip is emptied completely gently pull the tip along the side the vessel maintaining the angle from b) while pressing down the push-button to the final stop.
d) Allow the push-button to return to the top stop position.

5.1.3. Handling, Care and Maintenance:

a) When attached to a tip (filled or empty) do not lay down the pipette horizontally.
b) Avoid allowing a temperature difference between pipettes, tips and liquid as this may lead to incorrect dispensing volumes.
c) Do not allow any liquid to enter the pipette.
d) Do not clean the pipette with aggressive solutions (acetone, DCM, etc.)

5.2. Calibration of a Variable Volume Pipette

For each pipette to be calibrated three volumes will be tested:

- The nominal volume,
- Approximately 50% of the nominal volume,
- The lower limit of the useful volume range, or 10 % of the nominal range (whichever is greater)

5.2.1. Place the test liquid from the water container in the weighing vessel to a depth of at least 3 mm.

5.2.2. Record the temperature of the test liquid to the nearest 0.2 °C, T_1, the barometric pressure in the room to the nearest 1 kPa and the relative humidity to the nearest 10 %.

5.2.3. Select the test volume, this setting should not be altered during the text cycle of 10 measurements.

5.2.4. Prepare the piston pipette and the test cycle as follows:

a) Fit the selected tip to the piston pipette.

b) Fill the tip with test liquid and expel to waste five times to reach a humidity equilibrium in the dead air volume of the air-displacement piston pipette.
c) Place the weighing vessel with its added water on the balance pan.

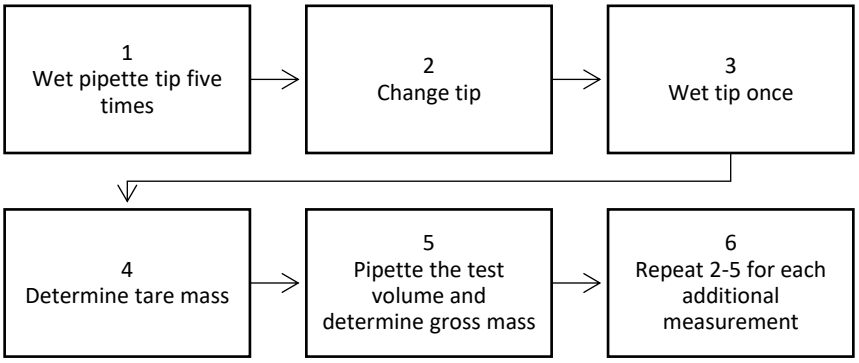
5.2.5. Perform the following test cycle:

a) Replace the disposable tip of the piston pipette.
b) Fill the piston pipette with test liquid, immersing its delivery orifice to an appropriate depth (see 5.1.1 c).
c) Expel the water to waste in order to pre-wet the tip and refill the piston pipette as described in b).
d) Tare the balance to zero, m_0.
e) Start the timing device
f) 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 10° to 45°
g) Use the blow-out feature of the piston pipette to expel the last drop of liquid 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.

Note: If it is necessary to remove the weighing vessel from the balance pan to permit delivery of the dispensed volume, avoid excessive handling and possible contamination by using lint-free gloves. Return the weighing vessel to the balance pan after delivery.

h) Record the mass of the quantity delivered.

Figure 1. Scheme of test procedure for piston pipettes with air interface (modified from ISO8655-6:2002)



5.2.6. Repeat the test cycle described in 5.2.5 until 10 measurements have been recorded as a series of masses m_1 to m_{10}.

5.2.7. Note the time to the nearest second taken to complete the 10 test cycles.

5.2.8. After the last weighing of 5.2.6 leave the weighing vessel on the balance pan for the time measured in 5.2.7 and record its mass, m_{11}. If the test volume is > 50 µL omit steps 5.2.7 and 5.2.8, as a correction for evaporation is unnecessary.

Note: If the weighing vessel was removed from the balance pan to enable delivery, leave it on the pan for half the time in 5.3.7 and then remove it from the balance and allow it to stand on the workbench for half the time measured in 5.3.7.

5.2.9. Measure the temperature of the remaining test liquid to the nearest 0.2 °C, T_2.

5.2.10. Evaluate the values obtained according to section 6. Ensure pipettes conform within the maximum permissible errors according to ISO8655-2:2002.

6. Evaluation

6.1. Calculation of Mean Test Temperature ($\bar{T}$)

(1) $\bar{T} = \frac{T_1+T_2}{2}$

where: T_1 is the initial temperature (5.2.2) and T_2 is the final temperature (5.2.9)

6.2. Calculation of Mass Loss in each cycle (L)

(2) $L = \frac{m_{10}-m_{11}}{10}$

where: m_{10} is the mass of final pipetted volume (5.2.6) and $m11$ is the mass following specified wait time (5.2.7)

6.3. Calculation of the Corrected Mass of each Quantity Delivered (m_c)

(3) $m_c = m_i + L$

where: m_i can be any mass value from m_1-m_{10} for a given cycle (5.2.6) and L is the mass loss for that cycle

6.4. Conversion of the Corrected Mass to Volume (V_i)

(4) $V_i = m_i \times Z$

where: *Z* is the temperature specific correction factor (appendix B)

6.5. Calculation of Mean Volume ($\overline{V}$)

(5) $\overline{V} = \frac{\sum_{i=1}^{n} V_i}{n}$

6.6. Systematic Error of Measurement (e_s)

(6) $e_s = \frac{100(\overline{V} - V_s)}{V_o}$

where: V_S is test volume used and V_0 is the nominal volume of the pipette

6.7. Random Error of Measurement (s_r)

(7) $s_r = \sqrt{\frac{\sum_{i=1}^{n}(V_i - \overline{V})^2}{n-1}}$

6.8. Uncertainty of Measurement (u)

Assuming the uncertainty resulting from the measurement of the delivered volume by the gravimetric method is small compared to the uncertainty resulting from the delivery process itself as related to the apparatus the uncertainty assessing the delivered volume at the 95 % confidence level may be calculated as follows: [¥]

(8) $u = |e_s| + 2s_r$

[¥] If a more detailed consideration is necessary, e.g. for very small volumes or for delivering apparatus with very high precision, this simplification is no longer valid, and a complete evaluation of the combined uncertainty must be made. A detailed description of this procedure may be found in ISO/TR 20461.

6.9. Maximum permissible error (piston driven pipettes)

Please see Appendix C.

Appendix A

Table A.1 Minimum requirements for balance (reproduced from ISO 8655-6:2002)

Selected volume[a] of apparatus under test	**Resolution**	**Repeatability and linearity**	**Standard uncertainty of measurement**
V	mg	mg	mg
1 µL ≤ *V* ≤ 10 µL	0.001	0.002	0.002
10 µL < *V* ≤ 100 µL	0.01	0.02	0.02
100 µL < *V* ≤ 1000 µL	0.1	0.2	0.2
1 mL < *V* ≤ 10 mL	0.1	0.2	0.2
10 mL < *V* ≤ 200 µL	1	2	2

[a] For practical purposes, the nominal volume may be used to choose the balance.

Appendix B

Table B.1 *Z* correction factors for distilled water as a function of test temperature and air pressure (reproduced from ISO 8655-6:2002)

Temperature (°C)	Air Pressure (kPa)						
	80	**85**	**90**	**95**	**100**	**101.3**	**105**
15.0	1.0017	1.0018	1.0019	1.0019	1.0020	1.0020	1.0020
15.5	1.0018	1.0019	1.0019	1.0020	1.0020	1.0020	1.0021
16.0	1.0019	1.0020	1.0020	1.0021	1.0021	1.0021	1.0022
16.5	1.0020	1.0020	1.0021	1.0021	1.0022	1.0022	1.0022
17.0	1.0021	1.0021	1.0022	1.0022	1.0023	1.0023	1.0023
17.5	1.0022	1.0022	1.0023	1.0023	1.0024	1.0024	1.0024
18.0	1.0022	1.0023	1.0023	1.0024	1.0025	1.0025	1.0025
18.5	1.0023	1.0024	1.0024	1.0025	1.0025	1.0026	1.0026
19.0	1.0024	1.0025	1.0025	1.0026	1.0026	1.0027	1.0027
19.5	1.0025	1.0026	1.0026	1.0027	1.0027	1.0028	1.0028
20.0	1.0026	1.0027	1.0027	1.0028	1.0028	1.0029	1.0029
20.5	1.0027	1.0028	1.0028	1.0029	1.0029	1.0030	1.0030
21.0	1.0028	1.0029	1.0029	1.0030	1.0031	1.0031	1.0031
21.5	1.0030	1.0030	1.0031	1.0031	1.0032	1.0032	1.0032
22.0	1.0031	1.0031	1.0032	1.0032	1.0033	1.0033	1.0033
22.5	1.0032	1.0032	1.0033	1.0033	1.0034	1.0034	1.0034
23.0	1.0033	1.0033	1.0034	1.0034	1.0035	1.0035	1.0036
23.5	1.0034	1.0035	1.0035	1.0036	1.0036	1.0036	1.0037
24.0	1.0035	1.0036	1.0036	1.0037	1.0037	1.0038	1.0038
24.5	1.0037	1.0037	1.0038	1.0038	1.0039	1.0039	1.0039
25.0	1.0038	1.0038	1.0039	1.0039	1.0040	1.0040	1.0040
25.5	1.0039	1.0040	1.0040	1.0041	1.0041	1.0041	1.0042
26.0	1.0040	1.0041	1.0041	1.0042	1.0042	1.0043	1.0043
26.5	1.0042	1.0042	1.0043	1.0043	1.0044	1.0044	1.0044
27.0	1.0043	1.0044	1.0044	1.0045	1.0045	1.0045	1.0046
27.5	1.0045	1.0045	1.0046	1.0046	1.0047	1.0047	1.0047
28.0	1.0046	1.0046	1.0047	1.0047	1.0048	1.0048	1.0048
28.5	1.0047	1.0048	1.0048	1.0049	1.0049	1.0050	1.0050
29.0	1.0049	1.0049	1.0050	1.0050	1.0051	1.0051	1.0051
29.5	1.0050	1.0051	1.0051	1.0052	1.0052	1.0052	1.0053
30.0	1.0052	1.0052	1.0053	1.0053	1.0054	1.0054	1.0054

Appendix C

Maxium permissible errors (piston-driven pipettes)

Nominal volume	Maximum permissible systematic error		Maximum permissible random error	
µl	± %	± µl [a]	± % [b]	± µl [c]
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[d]	0,3[d]
200	0,8	1,6	0,3[d]	0,6[d]
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

[a] Expressed as the deviation of the mean of a tenfold measurement from the nominal or selected volume (see ISO 8655-6:2002, 8.4).

[b] Expressed as the coefficient of variation of a tenfold measurement (see ISO 8655-6:2002, 8.5).

[c] Expressed as the repeatability standard deviation of a tenfold measurement (see ISO 8655-6:2002, 8.5).

[d] For piston pipettes of type D1 the maximum permissible errors may be ± 0,4 %.