MSL Technical Guide 30

Calibrating Piston Pipettes

Introduction

Piston pipettes are used extensively in a variety of applications for transferring known volumes of liquid for example; for drug testing in the pharmaceutical industry, for DNA profiling for forensics, for medical diagnostics such as blood testing, for environmental testing for pesticides in soil, and for food testing for chemical or biological contaminants. Through advances in technology, piston pipettes are now available for volumes from below 1 µL to above 10 mL and with repeatabilities and uncertainties as small as 0.05 µL. These advances have enabled tests on smaller samples and consequently reduced testing material costs.

This technical guide addresses the calibration of piston pipettes for dispensing volumes from 1 µL to 10 mL or more, including both fixed volume and variable volume piston pipettes. With variable volume piston pipettes, the dispensed volume is user-selectable over a range, for example 20 µL to 200 µL in 0.2 µL steps, and the maximum selectable volume is known as the nominal volume. For calibrating volumes larger than 10 mL, MSL Technical Guide 17 "Measuring Volume by Weighing Water" [1] may be used.

While largely based on the documentary standard BS EN ISO 8655:2002 "Piston-operated volumetric apparatus", parts 1, 2 and 6 [2, 3, 4], this technical guide is written for New Zealand ambient conditions. It includes a more appropriate uncertainty evaluation than is given in ISO 8655-6:2002 [4] or PD ISO/TR 20461:2000 [5], particularly in its treatment of balance linearity errors.

Piston Pipettes

Typically, piston pipettes – both fixed and variable volume – have best accuracies of about 0.6 % or 0.05 µL and best repeatabilities of about 0.3 % or 0.04 µL, depending on the volume. This is shown in Table 1 which gives the maximum permissible systematic and random errors (MPSE and MPRE) for pipette types A and D1 as given in Table 1 of ISO 8655-2:2002 [3]. The systematic error is the deviation of the mean of 10 volume measurements from the nominal or selected volume, while the random error is the sample standard deviation of the 10 measurements. Full definitions are in Sections 8.4 and 8.5 of ISO 8655-6:2002 [4].

To conform to ISO 8655-6:2002 (8.4.2 conformity evaluation), the systematic and random errors shall not exceed the relevant maximum permissible values in Table 1. No allowance for measurement uncertainty is required by [4]. However, to follow good metrological practice, we recommend that the measurement uncertainty is taken into account in evaluating conformance to MPSE. Conformance to MPRE only requires that the random error does not exceed the MPRE.

Achieving the pipette performance in Table 1 requires careful use of pipettes along with regular maintenance and calibration. Pipetting is a skill that must be learnt in order to achieve consistent and accurate results. Care should be taken to operate the piston of a pipette in a smooth and regular manner. Piston pipettes are delicate measuring devices with moving parts that must seal. They must be regularly checked to make sure that they are functioning correctly. The tip holder should be examined for marks or distortion that might cause a leak between it and the tip and the action of the piston should be checked to ensure that it is smooth and positive. Note also that piston pipettes do wear out, and hence lose accuracy, with use.

Nominal volume	Maximum permissible systematic error (MPSE)		Maximum permissible random error (MPRE)	
µL	%	µL	%	µL
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
1000	0.8	8.0	0.3	3.0
2000	0.8	16	0.3	6.0
5000	0.8	40	0.3	15.0
10000	0.6	60	0.3	30.0

Table 1. Maximum permissible errors for pipette types A and D1 from ISO 8655-2:2002.

It is recommended that pipettes are recalibrated at least once a year but wherever possible, and for critical applications, every three to four months (Section 7.1 of [6]).

Calibrating Piston Pipettes – overview

In principle, the calibration of a piston pipette is relatively simple. The pipette is used to deliver a quantity of distilled water to a weighing vessel on a balance. The volume of this water is then calculated from the weight and density of the water and compared with the ex-

pected volume. For pipette volumes of 1 mL or more, pipette calibration is almost this simple.

There are two complications. First, the measured weight of water must be corrected for air buoyancy and second, for micropipettes – piston pipettes with delivered volumes ≤ 1000 µL – a significant quantity of the delivered water may evaporate before it can be weighed.

To minimise evaporation, we recommend the use of an evaporation trap as discussed below. The rate of evaporation could be measured and a correction applied but, for pipette volumes below about 50 µL, the influence of evaporation needs to be reduced to achieve the required accuracy. An alternative to an evaporation trap is to use a two pan microbalance. Each pan of the microbalance has a small pre-wetted absorbent pad with capacity to hold delivered water. The idea is that the two pads have the same area and hence lose water by evaporation at the same rate.

Calibrating Piston Pipettes – theory

An initial balance reading r_0 is recorded with a weighing vessel in place on the balance. The preloaded quantity of distilled water is then delivered from the pipette into the weighing vessel and a second balance reading r_1 is recorded. The equation relating the delivered volume V to the balance readings is

$$V = (r_1 - r_0 + m_E + m_L)\left(\frac{1}{dw - da}\right)\left(1 - \frac{da}{ds}\right) \qquad (1)$$

where; m_E is the mass of water that evaporated between recording the balance readings r_0 and r_1, m_L is the correction for balance non-linearity, dw is the density of distilled water at temperature t, da is the ambient air density, and ds is normally 8000 kg/m^3. This value is used for density ds because electronic balances are normally set up to correctly measure the mass of objects with a density of 8000 kg/m^3. The evaporation term m_E is a characteristic of the pipette calibration setup and ambient conditions and is determined experimentally. In practice, 5 or 10 volumes V of water are delivered from the pipette and averaged.

The density of pure water in kg/m^3 as a function of temperature t for the range 15 °C to 25 °C can be calculated from

$$dw = 1000.2075 + 0.005398\,t - 0.005278\,t^2 . \qquad (2)$$

This approximate equation follows [1] and [7] and gives density values that agree with measurements on locally distilled water to within 0.01 kg/m^3.

Ambient air density da in the piston pipette calibration laboratory can usually be taken as

$$da = 1.2 \text{ kg/m}^3 \qquad (3)$$

with negligible uncertainty [1]. However, for pipette calibrations at altitudes higher than 100 m, a correction is required. See Equation (4) in [1].

Usually the aim of the pipette calibration is to determine the volume delivered by the pipette at a reference temperature t_0 (typically 20 °C). This volume $V(t_0)$ is calculated from V using the equation

$$V(t_0) = V\left[1 + \gamma_V (t_0 - t)\right] \qquad (4)$$

where γ_V is the thermal coefficient of cubic expansion of the pipette. In practice, γ_V can normally be assumed to be zero.

Equipment

The main items of equipment required for pipette calibration are a suitable electronic balance, a liquid storage reservoir, an evaporation trap, one or more weighing vessels, a timing device and a thermometer. A hygrometer and a barometer are not usually required for pipette calibration in New Zealand (but see *7.* and *8.* below).

1. A suitable balance.

Balances used for pipette calibration are chosen so that they are sufficiently accurate to determine compliance with the MPSE and/or the MPRE of pipettes as required. The dominant uncertainty from the balance is repeatability. As we explain later, balance linearity is rarely significant and its influence on pipette calibration is easily included. With the approach given here, the standard uncertainty of measurement of the balance referred to in Table 1 of BS EN ISO 8655-6:2002 [4] is not required as balance resolution, repeatability and linearity are covered separately.

Table 2 gives the minimum requirements for balances as a function of pipette volume according to ISO 8655-6:2002 [4]. Note that a one microgram resolution balance is required for calibrating some pipettes which means that pipette calibration can be an expensive business. Balances used for pipette calibration need a draft shield that is easily opened and closed.

Nominal volume under test V	Resolution mg	Repeatability and linearity mg	Standard uncertainty of measurement 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 ≤ 100 mL	1.0	2	2

Table 2. Minimum requirements for balances used to calibrate pipettes.

2. A liquid storage reservoir.

This reservoir must be clean and large enough to hold sufficient distilled water for a series of calibrations. A glass vessel with a screw-top lid is preferred. It is best to fill this reservoir with distilled water and leave it in the laboratory to be used for the pipette calibrations so that it reaches thermal equilibrium with the room.

It is also useful to have a clean beaker or equivalent for the water that is used for filling the pipette. We typically use a beaker with a capacity of (250 or 500) mL.

3. An evaporation trap

As mentioned earlier, we recommend the use of an evaporation trap to help reduce the influence of evaporation on the pipette calibration. These are available commercially as an accessory for some balances. An evaporation trap is shown in Figure 1. The trap works by creating a humid region just above the weighing vessel, which reduces the evaporation of water from the weighing vessel.

4. A weighing vessel.

Several weighing vessels with different capacities may be useful for best performance. The shape of the weighing vessel should be such that the pipette tip can be brought into contact with the inside wall at an angle of between 30° and 45° during delivery of the distilled water and drawn (8 to 10) mm up the inner wall of the vessel to remove any water droplets. [More on this later.]

Normally a transparent (plastic or glass) weighing vessel is used so that the operator can see the tip of the pipette as it is brought into contact with vessel wall. A tall narrow vessel (about 3 to 1 height to width ratio) is preferred to help minimize evaporation. As shown in Figure 1, the weighing vessel may be conical. Suitable weighing vessels may be provided as part of a commercial evaporation trap kit.

Make sure that the weighing vessel is firmly located in the centre of the pan so that it does not move sideways or tend to tip over when the pipette tip is brought into contact with the inner wall of the weighing vessel. A modified balance pan may be needed to achieve this.

5. A timing device.

A stop watch or clock accurate to better than 1 s is sufficient. A hands-free device like a wall clock indicating to the second may be preferred. The timing device is used to measure test cycle times and evaporation rates.

6. A thermometer.

A calibrated thermometer with a resolution of 0.1 °C and an accuracy (expanded uncertainty) ≤ 0.2 °C is suitable for pipette calibration. The temperature sensing element must be in a probe that can be cleaned and that is suitable for immersion in water. A stainless steel sheathed probe is recommended. Make sure this thermometer is immersed to the recommended depth in the water. This may influence the choice of beaker.

7. A hygrometer.

A hygrometer is not essential for pipette calibration as the influence of variations in humidity on the delivered volume calculated using Equation (1) is negligible. But a hygrometer can be very useful for identifying low humidity conditions in which static electricity may affect the balance behaviour (test room humidity <50 %rh). ISO 8655-6:2002 [4] includes a hygrometer in the apparatus list and includes the measured test room humidity during calibration in the pipette calibration certificate.

8. A barometer.

As with the hygrometer, a barometer is not essential for pipette calibration in New Zealand as the influence of variations in atmospheric pressure on the delivered volume calculated using Equation (1) is normally negligible. The only exception is for pipette calibration laboratories that are at an altitude above sea level that is greater than about 100 m. In this case, a barometer may be needed to measure the local atmospheric pressure in order to determine a value for the air density da in (1). See part 7 of the section below on Sources of Measurement Uncertainty.

9. A computer and software.

We strongly recommend that a computer with appropriate software is used to capture balance readings and to calculate pipette calibration results. Proprietary software may be used for this purpose or you can use the MSL pipette volume calibration spreadsheet, which is presented here. At the simplest, balance readings can be captured via a USB interface - using "Terminal" software on a Windows computer – and copied to the spreadsheet.

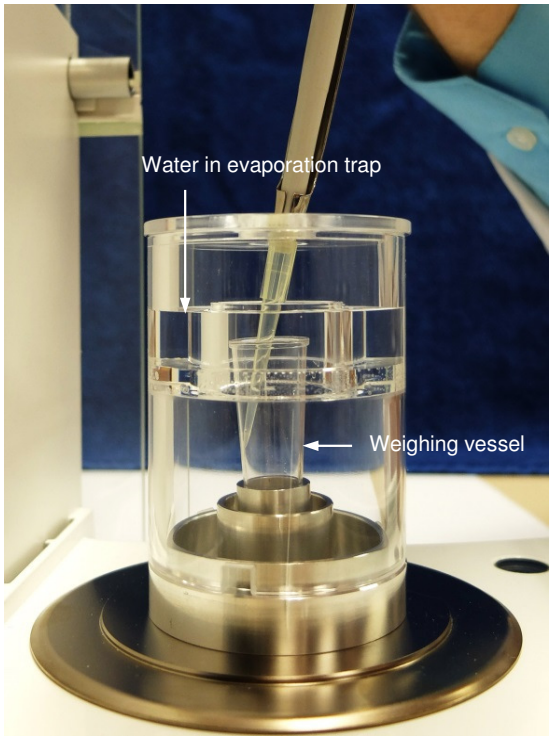

Figure 1. Electronic balance fitted with an evaporation trap and weighing vessel.

Room and Setup for Pipette Calibration

A suitable laboratory is required for pipette calibration. ISO 8655-6:2002 recommends that the laboratory is at a constant (± 0.5 °C) temperature between 15 °C and 30 °C and that the laboratory air has a relative humidity above 50 %rh. We recommend that the laboratory air temperature is controlled at 20 °C. In practice in New Zealand, there is normally no need to control the humidity although keeping the air above about 50 % rh helps avoid issues with static electricity.

The laboratory must have a solid bench for the balance that is wide enough to provide work space on both sides of the balance and preferably that is mounted on a solid floor. Rigid mounting is particularly important for higher resolution balances. Put a stand on the bench like that shown in Figure 2 to vertically support pipettes for calibration (on the right hand side of the balance if you pipette right-handed).

A warning: pipetting involves a lot of repetition that may lead to physical problems. The pipette calibration setup needs to be carefully considered to minimise the risk of repetitive strain injury (RSI).

Take precautions to avoid RSI. Ideally you will sit on a chair in front of the balance with your back upright, your feet flat on the floor and the bench at about elbow height. When pipetting, keep your wrist and forearm in as neutral a position as possible. Take regular breaks and perform stretching exercises. For more advice, search the web for "pipette RSI".

Pipette Calibration – step by step

The procedure given here is suitable for both fixed and variable volume (that is, user-selectable volume) piston pipettes and largely follows Section 7 of ISO 8655-6:2002 [4]. It is for piston pipettes designed "to deliver" rather than "to contain" so the calibration always involves dispensing water from the pipette into the weighing vessel.

1. Talk to the client

Talk to the client about their requirements. Confirm the volumes at which each pipette is to be calibrated, check what as-received measurements are required, if any, and ask how many measurements are required at each volume (usually 5 or 10). Note that 10 measurements at each volume are required to certify conformance to ISO 8655-6:2002 (section 7.1.2). For routine quality control, 5 measurements may be sufficient to meet accuracy requirements. A variable volume piston pipette is normally calibrated at three volumes; the nominal volume, approximately 50 % of the nominal volume and 10 % of the nominal volume (or the lower limit of the useful volume range if this is greater than 10 % of the nominal volume).

2. Check the pipettes

Check that pipettes submitted for calibration are clean and in good working order. Inspect the tip holder for any damage as this may cause leaks. Fit a matching tip and pipette some water to check that the mechanism moves smoothly and that there is no sign of a leak. If necessary, arrange with the client for the pipettes to be serviced or replaced.

3. Prepare for calibrating pipettes

Start preparing well before you intend to calibrate pipettes. Check that the balance has a current calibration certificate and that it includes linearity corrections and associated uncertainties. Make sure that the balance has been turned on (at least to standby mode). Obtain sufficient distilled or de-ionised water in the liquid storage reservoir and place this alongside the balance. Preferably, leave overnight to reach thermal equilibrium (and hence about 20 °C).

Set up the balance just before you start to calibrate pipettes. Adjust the scale factor of the balance using the CAL function. Check that the balance is performing as you expect by doing an accuracy check and a repeatability measurement at one or two loads using calibrated weights. These checks may be omitted if regular in-service checks are performed on the balance (see MSL Technical Guide 12 [8]). In addition, check the balance linearity as described in part 6 of the section below on Sources of Measurement Uncertainty.

Install the evaporation trap and an appropriate weighing vessel. Transfer water from the reservoir into the beaker. Using a pipette and water from the beaker, fill the evaporation trap nearly to the brim and the weighing vessel to a depth of at least 3 mm. Take care to avoid leaving water droplets on the wall of the weighing vessel - or remove them with a pipette. You may use a lid on the evaporation trap but it is not necessary.

4. Start calibrating pipettes

At this point, you are ready to start calibrating pipettes. As noted earlier, normally at least three volumes are tested for each variable volume pipette, including 10%, 50% and 100% of the nominal volume. The results are then used to calculate the accuracy and repeatability of the pipette at each volume. Conformance with the maximum permissible systematic and random errors given in Table 1 of ISO 8655-2:2002 may also be determined (see Table 1 above).

Figure 2. Stand for supporting pipettes.

The steps in the calibration of a pipette follow. For more detail, see Section 7 of ISO 8655-6:2002 [4] and the NPL Measurement Good Practice Guide on pipette calibration [6].

When calibrating piston pipettes, it is important that the time taken for the calibration measurements is consistent; that is the time taken for each of the steps in Section 4.3 below. This is so that subsequent dummy pipette calibrations (with no water delivered) can be used to reliably estimate the residual evaporation of water from the weighing vessel. One way to achieve this consistency is to follow a pre-planned time sequence. A second way is to use a consistent time to deliver the water from the pipette into the weighing vessel and to rely on the time from this point to a stable balance reading being about the same for each delivery.

4.1. Set the pipette to the test volume.

If the pipette has a variable volume, set the pipette to the volume to be tested. Check that the weighing vessel has water to a depth of at least 3 mm. If necessary, remove water from the weighing vessel with a pipette so that there is room for the water to be delivered.

Fit an appropriate tip to the piston pipette. Precondition the pipette by drawing water into the tip from the stock beaker and expelling it to waste (to establish humidity equilibrium in the dead air volume of the pipette). Do this five times for an air displacement pipette or once for a positive displacement pipette.

4.2. Record initial conditions.

Measure the initial temperature of the water in the beaker and the ambient temperature with a resolution of 0.1 °C. Enter these values into the analysis software or spreadsheet. If you have the appropriate instruments, also measure and record the ambient humidity and pressure.

4.3. Perform the calibration measurements.

a) Following ISO 8655-6, replace the disposable tip of the piston pipette.

b) Fill the piston pipette with water from the beaker, holding the pipette vertically and immersing the tip 2 mm to 3 mm below the water surface. Release the operating button slowly to fill the pipette and withdraw the pipette vertically and carefully from the surface of the water. Following ISO 8655-6, touch the delivery orifice against the side wall of the beaker.

c) Expel the water to waste in order to pre-wet the tip and refill the pipette as described in b) above.

d) Tare the balance and capture the initial balance reading r_0 at an appropriate time (for example zero seconds on the clock) or start the timing device. Re-charge the pipette.

e) Open the balance door.

f) Following 7.2.5 of ISO 8655-6, 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. Where applicable, use the blow-out feature of the pipette to expel the last drop of liquid before drawing the delivery end of the tip along the inner wall of the weighing vessel.

NOTE: There may be better ways of delivering the volume of water in the pipette into the weighing vessel.

See Section 8 below on Method Uncertainty.

g) Close the balance door.

h) Press the key to activate capture of a balance reading when it is stable (or capture the reading at a predetermined time).

i) Re-charge the pipette and repeat steps e) to h) for the second delivery at this volume.

j) Continue until the 5 or 10 volumes have been delivered and balance readings r_1 to r_5 or r_{10} have been recorded.

k) Record the time taken for the sequence of deliveries. Discard the used tip.

4.4. Record final conditions and balance readings.

Measure and record the final temperature of the water in the beaker, the ambient temperature and, if applicable, the ambient humidity and pressure. Enter these values into the analysis software or spreadsheet.

5. Analyse the measurements

Analyse the measurements using the MSL Pipette Volume Calculator shown in Figure 3 or use alternative software. The MSL Pipette Volume Calculator is an Excel spreadsheet and is available on request. For this Calculator, pale blue cells are for data entry while the values in pale orange cells are calculated. A drop-down box allows the nominal pipette volume to be selected. This also sets the MPSE and MPRE. The Calculator can accommodate 5 or 10 measurements at each volume. It calculates the average delivered volume from the weighing results, the associated measurement uncertainty and the systematic and random errors. It also tests for conformance with MPRE and MPSE (the latter, with and without measurement uncertainty). Check that the values entered for linearity and evaporation corrections are correct.

The uncertainties associated with the results are discussed in the following section.

MSL Pipette Volume Calculator				Date:	30/09/2015
Version:	20-Jun-16			Pipette ID:	240930058
				Client:	MSL
				Balance:	ME36S
			Linearity correction/ ug/g:		4.7
			Nominal Volume /uL:		**20**
	Water temperature /°C	Air temperature /°C	Air pressure /hPa	Air humidity /%rh	Start Time /s
Initial:	20.4	20.8	1013.1	38.0	0
Final:	20.5	20.8	1013.0	38.0	232
Mean:	20.5	20.8	1013.0	38.0	
	Evaporation correction: g per volume delivered				0.0000075
Measurements		Delivered water /g			
Delivered volumes	Balance reading /g	Mass increase /g	Evaporation corrected /g	Evaporation & Linearity corrected /g	Calculated volume uL
0	0.000000				
1	0.020006	0.020006	0.020014	0.020014	20.073
2	0.039878	0.019872	0.019880	0.019880	19.938
3	0.059770	0.019892	0.019900	0.019900	19.958
4	0.079655	0.019885	0.019893	0.019893	19.951
5	0.099556	0.019901	0.019909	0.019909	19.967
6	0.119469	0.019913	0.019921	0.019921	19.979
7	0.139402	0.019933	0.019941	0.019941	19.999
8	0.159320	0.019918	0.019926	0.019926	19.984
9	0.179225	0.019905	0.019913	0.019913	19.971
10	0.199207	0.019982	0.019990	0.019990	20.048
		Mean	0.019928	0.019929	19.987
	Standard deviation		0.000043	0.000043	0.0427560
Volume repeatability				MPRE /uL:	0.1
				Random error /uL	0.0427560
				Conformance with MPRE	**Yes**
Volume calculation			Total time for pipette deliveries /s		232
			Air density	kg/m^3	1.197
			Water temperature	°C	20.5
	Number of volume deliveries averaged *n*				10
	Mean corrected mass of delivered water			g	0.019928
				MPSE /uL:	0.2
				Calculated mean volume /uL	19.9870
				Deviation from nominal /uL	-0.0130
				Expanded uncertainty /uL	0.0313
	Conformance with MPSE - allowing for uncertainty				**Yes**
	Conformance with MPSE - no allowance for uncertainty				**Yes**

Figure 3. MSL Pipette Volume Calculator.

Sources of Measurement Uncertainty

The MSL pipette volume calculator includes an uncertainty calculation as shown in Figure 4.

While the largest uncertainty in piston pipette calibration is usually the repeatability of the delivered volume, other contributions to the uncertainty may include; thermometer calibration, measurement of the water tem-

perature, evaporation, repeatability of the delivered quantity, balance repeatability, balance resolution, balance linearity, variations in air density and an uncertainty associated with the method.

The treatment of measurement uncertainties given here follows the Guide to the Expression of Uncertainty in Measurement, now JCGM 100:2008 [9].

Volume uncertainty calculation									
Nominal volume /ul	20	**Type**	**Units**	**Value**	**Uncertainty type or distribution**	**Divisor**	**Standard uncertainty in influence quantity $u_i(x)$**	**Degrees of freedom ν_i**	**Equivalent standard uncertainty in volume /uL $u_i(y)$**
	Influence quantity								
Water temperature - thermometer calibration		B	°C	0.2	Expanded Uncertainty	2.0	0.100	50	0.000424
Water temperature measurement		B	°C	0.3	Rectangular full width	3.46	0.0867	20	0.000368
	Evaporation	A	g	0.0000028	Normal	1	0.0000028	54	0.002808
Repeatability of delivered quantity SEOM		A	g or mL	0.000013	Normal	1	0.0000135	9	0.013521
	Balance repeatability	A	g	0.000001	Normal	1	0.0000009	9	0.000951
	Balance resolution	B	g	0.000001	Rectangular full width	3.46	0.0000003	50	0.000290
Assuming constant air density (1.2 kg/m^3)		B	kg/m^3	0.012	Normal	1	0.012	50	0.000211
	Linearity	B	ug/g	200.0	Expanded Uncertainty	2	0.002	50	0.000002
	Other								
						Combined standard uncertainty u_C /uL			0.013858
						Effective degrees of freedom ν_{eff}			9.9
							Coverage factor k		2.26
							Expanded uncertainty U_C /uL		0.031

Figure 4. MSL Pipette Volume Uncertainty Calculation.

1. *Thermometer calibration*

The calibration certificate for the thermometer that is used to measure the water temperature will give the uncertainty associated with its calibration. For example, the certificate might give an expanded uncertainty of 0.2 °C with a 95% level of confidence and a coverage factor *k* (divisor) of 2.0. The divisor converts the expanded uncertainty to a standard uncertainty. This information is entered into the pale blue cells in the uncertainty calculation as shown in Figure 4. Here we assume ν = 50 degrees of freedom indicating a high level of confidence in the uncertainty value

2. *Water temperature measurement*

The uncertainty associated with measuring the temperature of the water used in the pipette calibration can be estimated from the range of measured temperatures for the water in the beaker during pipette calibration, for example 0.3 °C. This is entered into the uncertainty calculation as the full width of a rectangular distribution so the divisor to convert it to a standard uncertainty is √12 = 3.46. Degrees of freedom are somewhat arbitrarily ν = 20. The density of water decreases by about 0.206 kg/m^3 per °C in the temperature range 15 °C to 25 °C. For pipettes, this corresponds to a volume change of about 0.02 % per °C.

3. *Evaporation*

Using an evaporation trap significantly reduces the loss of water by evaporation but there will still be some residual evaporation. This is a characteristic of the pipette calibration setup.

At least one dummy calibration is performed along with normal pipette calibrations to assess the residual evaporation as it may vary from day to day. For a dummy calibration, the normal pipette calibration process is followed as detailed in Section 4.3 but no water is delivered into the weighing vessel. For each dummy calibration, the residual evaporation is the mean of the 10 "deliveries" and the associated standard uncertainty is the standard deviation of the 10 values.

The evaporation correction is entered into the pipette volume calculator and the standard uncertainty associated with this correction goes into the volume uncertainty calculation. For example, six dummy calibrations using the facility described here gave a mean evaporation of 7.5 µg per delivery with a standard uncertainty (standard deviation) of about 2.8 µg. This uncertainty has a normal distribution with a divisor of 1 and ν = 54.

4. *Repeatability of delivered volume*

The repeatability of the delivered volume is calculated following Section 8.5.1 of ISO 8655-6 as

$$s_r = \sqrt{\frac{\sum_{i=1}^{n}\left(V_i - \bar{V}\right)^2}{n-1}} \,. \qquad (5)$$

This is the sample standard deviation where V_i is the i'th measured volume and $\bar{V}$ is the average of the n measured volumes.

5. *Balance resolution and repeatability*

As discussed in the section on equipment, the balance used for pipette calibration is chosen so that it is sufficiently accurate to determine compliance with the MPSE and/or the MPRE (Table 1). Uncertainties associated with balance repeatability and resolution are included in the uncertainty calculator. The value of the resolution is entered as the full width of a rectangular distribution so the divisor to convert it to a standard un-

certainty is $\sqrt{12} = 3.46$ and $\nu = 50$. For balance repeatability, the standard deviation of 10 repeat measurements is entered with $\nu = 9$. The value for repeatability may be taken from recent in-service checks or from repeatability measurements recorded during the setup for pipette calibration. Repeatability may be measured near mid or full capacity of the balance or in the sub-range used for pipette calibration as the repeatability of an electronic balance usually changes little with load.

6. Balance linearity

Deviations of the electronic balance scale from linearity are rarely significant for pipette calibration, but are included in the uncertainty calculation for completeness. Figure 5 shows the linearity correction versus load for a 1 µg resolution balance (blue line). The dashed line shows the linearity correction for the 24 g to 30 g range used for pipette calibration. This balance non-linearity appears to be large but in fractional terms the linearity correction is only 3.7 µg/g.

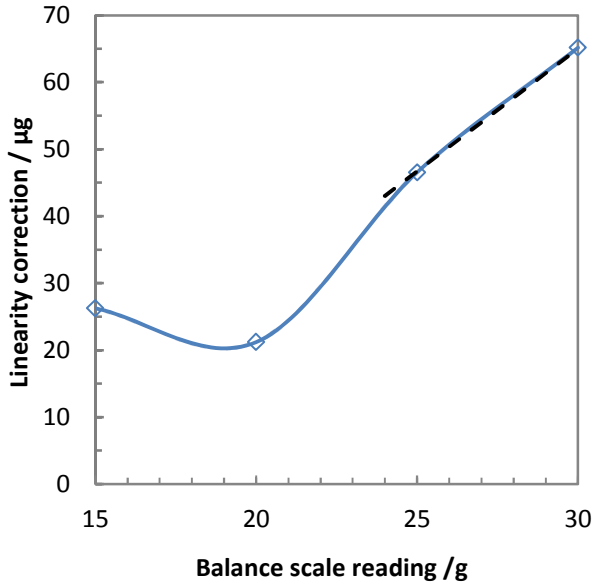

Figure 5. Example of balance linearity correction.

The uncertainty associated with balance linearity is usually much larger than the linearity correction itself! For the 1 µg resolution balance discussed here, a 2 g or 5 g weight would be sufficient for the linearity measurement, along with trim weights to change the base load to which the 2 g weight is added. Calibrated weights are recommended for checking the balance linearity and these should comply with OIML R 111-1 [10], Class F_2 or better. For example, a 2 g Class F_2 weight will be accurate to about 400 µg, which in fractional terms is 200 µg/g as in Figure 4.

7. Variations in air density

Equation 1, which relates the delivered volume V to the weight of water delivered from the pipette, includes the air density da. In practice, variations in air density have a very small influence on pipette calibrations when the ambient temperature is in the range 15 °C to 25 °C, and with ambient pressure variations at sea level typical of New Zealand (990 hPa to 1040 hPa). For this situation, we allow an uncertainty of 0.012 kg/m^3 as the standard deviation of a normal distribution with a divisor of 1 and $\nu = 50$. If you are calibrating pipettes at an altitude above about 100 m, then apply a correction to da following Equation (4) in [1] and/or contact us for advice.

8. Method uncertainty

Inter-laboratory comparisons of pipette calibration following the gravimetric method specified in ISO 8655-6 have revealed inconsistencies between results. These differences are not explained by known sources of measurement uncertainty [11]. As a consequence, a method uncertainty u_{Method} has been postulated for ISO 8655-6. As shown in Table 3, u_{Method} is between 0.18 and 0.30 times the MPSE when expressed as a standard uncertainty [11].

This method uncertainty is not included in the MSL pipette volume uncertainty calculation but should be taken into account when establishing the lower limit to the achievable measurement uncertainty for pipette calibration. For example, for a 2 µL pipette with an MPSE of 0.08 µL, following Table 3, the standard uncertainty due to the method is u_{Method} = 0.18 x 0.08 µL = 0.0144 µL or an expanded uncertainty of 0.029 µL.

The origin of the method uncertainty is likely to be associated with the procedure used to deliver the contents of the pipette into the weighing vessel.

Nominal volume /µL	u_{Method}/MPSE	Nominal volume /µL	u_{Method}/MPSE
2	0.18	200	0.18
5	0.18	500	0.17
10	0.27	1000	0.17
20	0.25	2000	0.17
50	0.19	5000	0.20
100	0.20	10000	0.30

Table 3. Method standard uncertainty as a fraction of MPSE for volumes from 2 µL to 10 mL from [11].

The procedure given in ISO 8655-6 is not ideal. Touching the pipette against the wall of the weighing vessel disturbs the balance. Drawing the pipette tip along the wall of the weighing vessel disturbs the balance even more. Touching the pipette tip against the weighing vessel wall with the pipette at a 30° to 45° angle is not practical in many pipette calibration setups.

A more nearly ideal procedure is to deliver the contents of the pipette into the weighing vessel with the pipette vertical and with the pipette tip close to (one or two mm above) the water surface. This procedure relies on good quality pipette tips that do not retain any visible water when the pipette is discharged. Hydrophobic pipette tips are available and may be useful. MSL plans to investigate these issues.

Summary & Comments

This technical guide addresses the calibration of piston pipettes for dispensing volumes from 1 µL to 10 mL or more. It is largely based on ISO 8655 with a focus on New Zealand conditions and with an improved uncertainty evaluation.

Calibration of piston pipettes is complicated at present by work that has identified variations in results that

cannot be explained by current uncertainty evaluations. Some thoughts have been offered on the source of the variations and further work is planned.

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Further Information

If you want to know more about volume measurement, density measurement, balances or weighing, contact MSL at msl@callaghaninnovation.govt.nz or visit the MSL website at http://msl.irl.cri.nz/.

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