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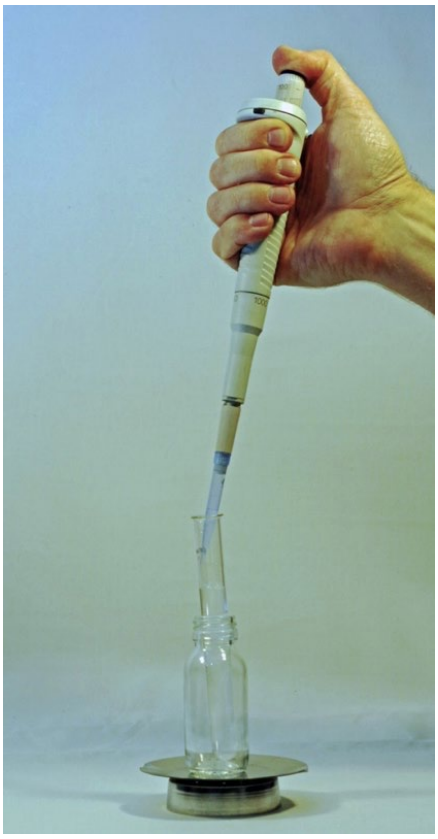
In-house calibration and control of piston operated pipettes



By

Martin Jönsson and Bertil Magnusson
Eurolab Eurachem Sweden

Nordtest
Slettetoften 9,
DK-2630 Taastrup

Telephone +45 21 21 44 44
nordtest@nordtest.info
www.nordtest.info

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Edition 1.1 (2025)

Martin Jönsson and Bertil Magnusson
Eurolab Eurachem Sweden

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Abbreviations and Symbols

The following symbols and abbreviations occur frequently in this guide. Other symbols and abbreviations are defined on first use.

Abbreviations

POVA	Piston operated volumetric apparatus
VIM	International Vocabulary of Metrology

Symbols

U	Expanded measurement uncertainty in %
s	Standard deviation
b	Relative bias in %
%RSD	Relative standard deviation in %
AL	Action limit
Z	Factor for conversion from mass to volume of pure water

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1 Introduction

Volumetric apparatus are commonly used in laboratories for addition of specific amounts of liquids such as reagents, solvents and samples. The specification of performance of the apparatus can be obtained from the supplier or given in a certificate. Apparatus that can be regarded as stable over time do not normally need any verification of the specification; for example Class A Borosilicate volumetric glassware calibrated at 20 °C has a thermal and chemical resistance and the volume stated is retained over a long working lifetime if no degradation is observed [1][*]. The specification for volumetric apparatus with moving parts, such as piston operated pipettes, need to be verified. This can be performed by comparing the delivered volume of pure water using the pipette with a volumetric reference value. The reference value is obtained by weighing the delivered amount and calculating the volume by multiplying the mass with a factor *Z*.

This document describes calibration and quality control procedures of piston operated pipettes, in this document referred to as pipettes. Important references are;

- ISO 8655-1 [3] giving general requirements and terms for volumetric measuring apparatus;
- ISO 8655-2 [4] and ISO 8655-6 [5] describes gravimetric methods for determining the volume delivered from piston operated volumetric apparatus; and
- ISO 8655-10 [6] giving user guidance.

2 Scope

This Nordtest technical report presents three procedures for piston operated volumetric apparatus used in routine laboratories:

1) in-house calibration;

2) verification of specification; and

3) routine check.

Detailed guidance is presented for pipettes for volumes from 10 µL to 10 mL but the procedures presented here are also applicable to burettes, dilutors, dispensers and manually operated laboratory syringes.

[*] e.g. UKAS [2] identify a number of scenarios where glassware can be used without prior calibration, for example Class A volumetric flasks with a tolerance of ± 0.2 % can be used directly without prior calibration as long as the capacity of the flasks are at least 25 ml. Further information about volumetric glassware is available in the UKAS document.

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3 Terms and definitions

3.1 Testing (of a pipette)

A set of operations that establish the relationship between the delivered volume and the corresponding target volume of the apparatus with or without the estimation of the measurement uncertainty, ISO 8655-1 [3].

3.2 Calibration

Operation that, under specified conditions, in a first step, establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and in a second step, uses this information to establish a relation for obtaining a measurement result from an indication, VIM 2.39 [7].

Comment: This complicated term is further explained in the Eurachem Guide Terminology in Measurement [9]. *In this report the values obtained from the balance represent the measurement standard and the set volume of the pipette represents the indication.*

3.3 In-house calibration

Calibration by a laboratory of equipment used internally such as thermometers, and pipettes.

3.4 Piston operated volumetric apparatus (POVA)

Devices used to aspirate or deliver specific volumes of liquids. These can be manually or semi-automatically operated and are controlled by mechanical, electromechanical or electronic means, ISO 8655-1 [3]. In addition to pipettes, burettes, dilutors, dispensers and syringes are examples of devices described in ISO 8655-1.

3.5 Pipettes

Pipettes are used to aspirate and deliver liquids. Pipettes can be fixed or with variable volume, ISO 8655-1 [3]. The piston operated pipette can be of air displacement type, having a body of air between the piston and the liquid or positive (direct) displacement type with the piston in direct contact with the liquid.

3.6 Target volume

The volume chosen for a variable volume pipette. For a fixed volume pipette, the target volume is equal to the nominal volume, ISO 8655-1 [3].

4 Equipment

4.1 Pure water

Pure water with a conductivity < 0.5 mS/m (> 2 Mohm·cm) as stated for Grade 3 in ISO 3696 [10].

4.2 Analytical balance

Analytical balance with resolution 0.01 mg or better.

Comment: For volumes smaller than 20 µL ISO 8655-6 [5] recommends a resolution of 0.001 mg.

4.3 Thermometer

Thermometer with a resolution of 0.1 °C and an expanded uncertainty of 0.2 °C as stated in ISO 8655-6 [5].

4.4 Container for weighing (weighing vessel)

The weighing vessel should be chosen taking into account the evaporation loss of water during the delivery and weighing procedure. A weighing vessel with narrow glass neck can be used. Alternatively, a longer test tube kept in an upright position with suitable support, see Figure 1.

4.5 Pipette and suitable pipette tips

According to ISO 8655-2 [4] the tip shall be made of material giving consistent results. The performance parameters determined (as bias and repeatability) are specific for the unique combination of an individual pipette and tip type.

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5 Procedure

5.1 General

Three different procedures for pipettes are presented here:

1. In-house calibration;
2. Verification of specification;
3. Routine check.

The general testing of pipettes described in section 5.4 is the same for all procedures. The room temperature shall be 18 – 25 °C. All items, pipettes, tips, vessels and water, shall be at the current room temperature ± 0.5 °C according to ISO 8655-6 [5]. The test of the pipette is performed by weighing the delivered volume and calculating the volume using a factor *Z.* The factor is based on the density of pure water and the air buoyancy*. This gives metrologically traceable values to decide if the obtained volume is within tolerance or action limits.

A pipette is only accepted if the volume obtained after rounding is within the limits. For example, for a 1 mL pipette with limits 0.98 – 1.02 mL the volume obtained during testing shall be between 0.975 mL and 1.024 mL. If out of tolerance or action limits, service and cleaning is carried out and the pipette is verified again.

The frequencies should be documented in the laboratory's quality management system. The chosen time intervals can be based on historical data. The routine checks are performed frequently and can consist of individual tests. The time points for verification of specifications and for calibration can either be determined by set time intervals or be triggered by non-compliance in routine checks.

Guidance for use of pipettes are given by many manufactures. The standard ISO 8655 part 10 is *User guidance, and requirements for competence, training, and POVA suitability* [6]. A detailed study of factors that influence pipetting using micropipette was published in 2015 [8].

5.2 Variable volume pipettes

A variable volume pipette should preferably be tested first at the maximum volume, and then at approximately 50 % of the maximum volume, and finally at the lowest volume. A laboratory can limit the volume range over which a pipette is used. In such cases it is the restricted range which is tested. Set the volume and do not change it until all tests with the current volume have been completed.

5.3 Control charts used in this report

Control charts are used to determine if the performance of an instrument is within a specified tolerance and to monitor the variation over time. In this technical report a decision rule of simple acceptance is used – see further ILAC G8 [11]. In the control charts action limits (AL) are used. One result *outside* an action limit indicates that the pipette is not in control and action is needed. One single re-test is allowed to check that the first test was performed correctly. When the pipette is confirmed to be out of control common actions are to perform service and possibly adjustment. Before use a verification of the specification of the pipette is performed.

5.4 Testing of a piston operated pipette

This testing procedure is used for in-house calibration, verification of specification and routine check. It is useful to consult guides from the manufacture for best practice in the use of a specific pipette. Normally 10 aliquots are pipetted at each volume and the tip is changed to a new one after five aliquots. For a routine check one aliquot is pipetted.

* Weight loss due to air pressure – for water the weight loss is ca 0.1 %.

The weighing vessel shall preferably contain enough water to cover the bottom of the vessel when the testing is started. The following procedure is recommended for a manually operated pipette.

a. Note the temperature in the laboratory
b. Put the weighing vessel on the analytical balance
c. Attach the pipette tip and wet the tip by rinsing at least three times with the pure water
d. Press down on the plunger
e. Hold the pipette vertically (0°) and immerse the tip to a depth of about 3 - 5 mm below the surface
f. Release the plunger slowly
g. Hold for 1-2 seconds then raise the pipette from the liquid
h. Zero set the balance
i. Hold the pipette tip against the inside of the vessel with the pipette at a 30 - 45° angle* – Figure 1
j. Depress the plunger smoothly to the first stop to transfer the liquid. Where applicable, wait 1 second then use the 'blow out' or 'purge' function to transfer the last drop by pushing the plunger all the way down. Finish by pulling the tip towards the inside of the weighing vessel and removing the pipette
k. Note the mass m_i

If required repeat the cycle from c to j until all measurements are completed.

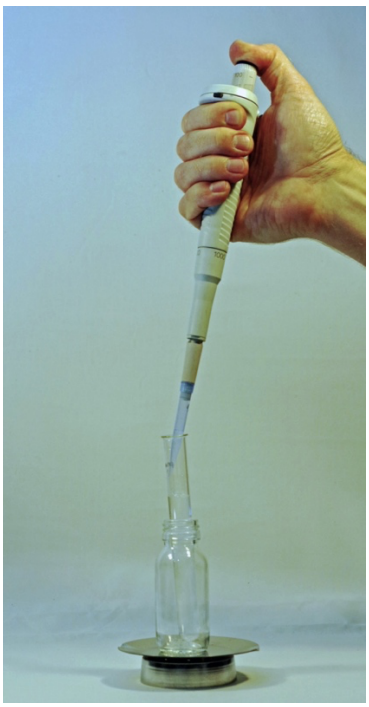

Figure 1 — Pipetting into a test tube placed on a balance
(Photo by Martin Jönsson)

Calculate the volume, V_i by multiplying the mass, m_i with the conversion factor Z; $V_i = m_i \times Z$. For pure water a value of 1.003 for Z can be used for normal conditions in the laboratory (18 - 23 °C and air pressure 95 - 105 kPa). Conversion factors with 4 decimals at specific temperatures are given in Table 1.

Table 1 — Conversion factors, *Z*, for pure water as a function of the temperature of the water at normal air pressure (101,3 kPa) ISO 8655-6 9.3.3 [5]

Temperature °C	***Z*** mL/g	**Temperature** °C	***Z*** mL/g
18.0	1.0025	22.0	1.0033
18.5	1.0026	22.5	1.0034
19.0	1.0027	23.0	1.0035
19.5	1.0028	23.5	1.0036
20.0	1.0029	24.0	1.0038
20.5	1.0030	24.5	1.0039
21.0	1.0031	25.0	1.0040
21.5	1.0032	25.5	1.0041
NOTE 1 The conversion factors take into account the air buoyancy correction of about 0.1 % based on air pressure at the corresponding test temperature. NOTE 2 The conversion factors given can be used for normal air pressure (95 – 105 kPa). For other pressures and temperatures see more detailed information in ISO 8655-6.			

* Tests show that at angle of 0° (vertical) there is a negative bias of about - 0.2 % of the delivered volume [8].

6 In-house calibration

A calibration includes measuring the bias and calculating the uncertainty of the measured bias. The calibration of the pipette is performed according to the test procedure in section 5.4 with ten replicates. The mean volume $\bar{V}$ and the %RSD of the 10 delivered volumes are calculated. The bias, *b* is calculated according to Equation 1.

$$b = \frac{\bar{V} - V_{ref}}{V_{ref}} \times 100 \qquad \text{Equation 1}$$

The relative uncertainty for the calibration can be calculated according to GUM [12] as described in section 13 in ISO/TR 20461 [13]. For in-house calibration we propose to use the simplified approach described in Annex A in ISO/TR 20461 for *a single delivery* to evaluate the relative uncertainty. In this approach, *U*, is calculated by combing the bias in percent, *b,* the coverage factor *k* (for 95 % confidence) and the repeatability, %*RSD* according to Equation 2.

$$U = |b| + k \times \%RSD \qquad \text{Equation 2}$$

6.1 Comments on the uncertainty calculations

Error sources contributing to the uncertainty of the volume delivered by a pipette are operator handling including repeatability, temperature, balance indication, water density, air pressure and for the pipette the cubic thermal expansion coefficient. The major contribution according to ISO/TR 20461 [13] is the repeatability. For a laboratory performing in-house calibrations the important outcome is the uncertainty *of a single delivered volume* and here the simplified approach proposed in ISO/TR 20461 Annex A can be used. Comments on this simplified approach:

1. Only a single delivered volume is considered. The evaluated uncertainty for a single delivered volume is useful when assessing the uncertainty for routine use in the laboratory.
2. The random error is the repeatability at 95% confidence, $k \times$ %RSD.*
3. The systematic error expressed as percent bias is added to the uncertainty, taking into account that no bias corrections is applied in daily use.

6.2 Requirements for in-house calibration

According to EA 04/02 [14] and ISO/IEC 17025 [15] the following shall be required for an accredited external calibration; competent personal, a calibration method, routines for uncertainty evaluation, documentation of the result of the calibration in a calibration certificate and participation in interlaboratory comparison.

For an in-house calibration this Nordtest report proposes that the following shall be required: competent personal, a calibration method, routines for uncertainty evaluation and documentation of the result of the calibration.

For in-house calibration participation in interlaboratory comparisons can be replaced by interlaboratory comparison activities for the specific test methods in which the internally calibrated

* The random error here is the repeatability for a single delivery. For the uncertainty of the mean delivery also the reproducibility is important to include according to ISO/TR 20461 [13].

equipment is used. If additional verification is required results from an in-house calibration can be compared with an external accredited calibration of the same pipette.

6.3 Example of an in-house calibration

In this example a maximum expanded uncertainty for a 1 ml pipette in a laboratory is set to 2 % for a single delivery. Testing of a 1 ml pipette according to section 5.4 gave a bias of -0.40 % and an RSD of 0.20 %. With ten replicates the coverage factor, k, is 2.27. The relative expanded uncertainty, U, for the volume of a *single delivery* of 1 mL using this pipette according to Equation 2 is:

$$U = |-0.40| + 2{,}27 \times 0.20 = 0.85\ \% \qquad \text{Equation 3}$$

The results from the in-house calibration of this 1 mL pipette over a longer time period are plotted in Figure 2.

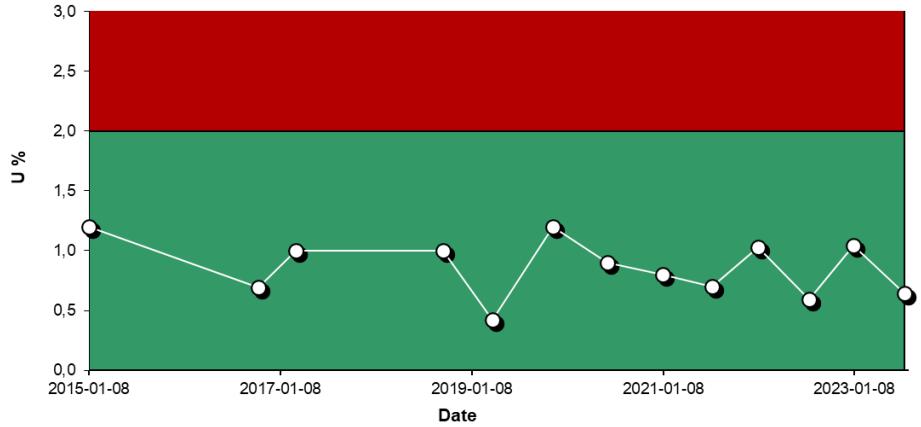

Figure 2 — X-chart with results over time from in-house calibrations — the expanded measurement uncertainties of single deliveries for a 1 ml pipette are shown with tolerance of 2 %

6.4 Example of tolerances used in a laboratory

The tolerances are set by the laboratory and can be wider than the specification – fit for the intended use. Examples of tolerances used in a laboratory for maximum bias and maximum repeatability is shown in Table 2. The maximum expanded uncertainty is calculated from bias and repeatability for a single delivery according to Equation 2.

Table 2 — Examples of maximum bias, repeatability and calculated uncertainty for piston operated pipettes y

Volume range (ml)	Max bias %	Max repeatability %	Calculated expanded measurement uncertainty %
≥ 0.010 to < 0.050	2	1.3	5
≥ 0.050 to < 0.100	2	0.9	4
≥ 0.100 to < 1.000	1	0.9	3
≥ 1.000 to ≤ 10.000	1	0.9	3
NOTE The expanded uncertainty for a single delivery is calculated from max bias and max repeatability according to Equation 2.			

7 Verification of specification

The limits can be set by the specification given by the manufacturer or the ones proposed in ISO 8655-2 [4]. The limits can also be set by the laboratory. Here is an example of using acceptance control charts for a verification of a fixed 1 ml pipette with the following specification set by a laboratory:

- bias max ± 1 %;
- random error max RSD 0.3 %.

The testing of the pipette is performed 10 times according to section 5.4.

The mean volume, $\bar{V}$ is calculated and the % bias, b is calculated as the difference from the target volume, V_{ref}, according to Equation 1. The calculated bias is compared with the specification.

The %RSD of the 10 delivered volumes is calculated and compared with the specification. The results from the verification of the internal specification over a longer time period are plotted in Figure 3.

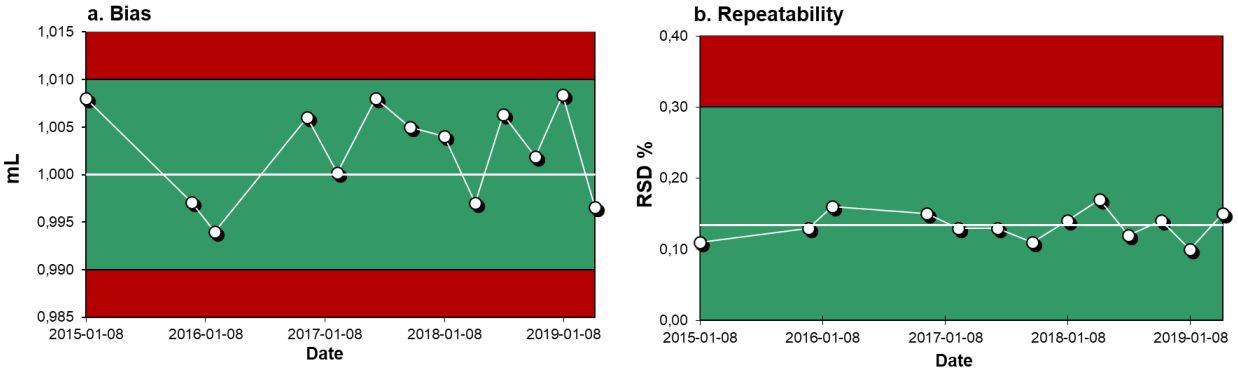

Figure 3 — X-charts with results over time from verifications of 1 ml pipette: a. bias (systematic error) with tolerance ± 0,01 mL and b. repeatability with a tolerance of 0.3 %RSD

8 Routine check

The limits can be set according to the requirements. In Table 3 is shown an example from a laboratory with action limits. The limits are set based on expanded uncertainties required from 5 to 2 % depending on the volume delivered.

Table 3 — Proposed action limits (AL) based on required uncertainty (*U*) of obtained volume

Volume µl	***U*** **%**	**Lower AL µl**	**Upper AL µl**
10	5	9.5	10.5
50	4	48.0	52.0
100	3	97	103
200	3	194	206
250	3	243	258
500	3	485	515
Volume mL	***U*** **%**	**Lower AL mL**	**Upper AL mL**
1	2	0.98	1.02
2	2	1.96	2.04
5	2	4.90	5.10
10	2	9.80	10.20
NOTE The calculated volume is rounded to the number of decimals given for the action limits – e.g. results for a 1 mL volume are rounded to 2 decimals before comparing with limits.			

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Return address:
Nordtest,
Slettetoften 9
DK-2630 Taastrup, DK

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