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QUALIFICATION OF EQUIPMENT

QUALIFICATION OF PISTON-OPERATED VOLUMETRIC APPARATUS (POVA)

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ANNEX 6 OF THE OMCL NETWORK GUIDELINE
"QUALIFICATION OF EQUIPMENT"

QUALIFICATION OF PISTON-OPERATED VOLUMETRIC APPARATUS (POVA)

Note: Mandatory requirements in this document are defined using the terms "shall" or "must". The use of "should" indicates a recommendation. For these parts of the text other appropriately justified approaches are acceptable. The term "can" indicates a possibility or an example with non-binding character.

Contents

1. Introduction

The present document is the 6th Annex of the core document "Qualification of Equipment" [1], and it should be used in combination with it when planning, performing and documenting the qualification of piston-operated volumetric apparatus (POVA).

The core document "Qualification of Equipment" contains the Introduction and general forms for Level I and II of qualification, which are common to all types of instruments.

Level III and IV testing must be carried out, as an ISO/IEC 17025 [2] requirement, by trained personnel.

This Guideline has been elaborated according to the standard ISO 8655 (Parts -1, -2, -5, -6, -7, -8 and -10) [3-9]. Requirements and (if applicable) corresponding acceptance criteria should be

applied; however, other appropriately justified deviations are acceptable provided they are traceable.

This document describes the method given in ISO 8655-6 "Gravimetric reference measurement procedure for the determination of volume" [6], which is the most commonly used.

Another reference measurement procedure is described in ISO 8655-8 "Photometric reference measurement procedure for the determination of volume" [8], which is applicable to apparatus with a maximum nominal volume of 5 000 µL.

Alternative procedures are given in ISO 8655-7 "Alternative measurement procedures for the determination of volume" [7].

In this Annex the general requirements for metrological confirmation of POVA, which comprises calibration and verification, are described, as well as other requirements included in ISO 8655-10 "User guidance, and requirements for competence, training, and POVA suitability" [9].

In cases other than calibration (e.g. routine tests), some permitted deviations from ISO 8655-6 [6] and ISO 8655-8 [8] requirements are described in this document.

2. Aim and Scope of the Guideline

This guideline describes the requirements for POVA used in chemical and biological tests irrespective of the mode of operation (manual or electronic).

Requirements and test procedures are based on the ISO 8655 standard.

The following types of pipettes have been considered in this guideline:

1. Fixed-volume single-channel pipettes (air displacement (type A) and positive displacement or direct displacement pipettes (type D, with a reusable or disposable plunger and capillary))
2. Variable-volume single-channel pipettes (air displacement (type A) and positive displacement or direct displacement pipettes (type D1) reusable or (type D2) disposable plunger and capillary))
3. Fixed-volume multi-channel pipettes (air displacement (type A))
4. Variable-volume multi-channel pipettes (air displacement (type A))
5. Electronic motorised pipettes
6. Dispensers (e.g. repetitive piston pipettes)

NOTES:
Although designed as variable-volume pipettes, electronic motorised pipettes may be used in different modes:

- Pipetting mode: the selected volume is aspirated and dispensed in forward mode pipetting.
- Multi-dispensing mode: the aspirated volume is dispensed repeatedly in a predefined number of aliquots, which can be of equal or different volumes.
- Mixing mode: pipetting step is followed by a mixing phase, composed of repeated aspirating and dispensing.
- Reverse mode: aspiration of the selected volume added with an extra amount of liquid that remains in the tip after the dispensing of the selected volume. The remaining volume can subsequently be discarded.
- Multi-aspiration: aspiration of a predefined number of equal or different volumes, followed by the dispense of the total aspirated volume.

Additionally, this guideline provides guidance, based on ISO 8655-10 [9] regarding:

- The selection of POVA (including exchangeable parts) and best practices for their use;
- User qualification/re-qualification (requirements for user training and competence);
- POVA performance requirements and tolerances to ensure fitness for their intended use;
- Replicate measurements and uncertainty.

3. Glossary (ISO 8655-1):

Calibration: set of operations that establish the relationship between the delivered volume and the corresponding selected volume of the apparatus with associated measurement uncertainties.

NOTE:
Calibration requires no operation which permanently modifies the apparatus and does not require adjustment of the device.

Routine testing: testing equipment monitoring or analytical quality control routines to be performed at regular intervals, for example, every three months. Other time intervals may be adopted giving due consideration to the factors described in Level IV (paragraph 8). This term is a synonym of "verification".

Measurement uncertainty: non-negative parameter, associated with the delivered volume, that characterises the dispersion of the volumes that could reasonably be attributed to the delivered volume based on the information used.

When performing calibration, the measurement uncertainty shall be evaluated and reported; however, in routine testing it is optional.

Metrological confirmation: set of operations required to ensure that the POVA conforms to the requirements for its intended use.

NOTE:

Metrological confirmation generally includes calibration and verification, and any necessary adjustment or repair, and subsequent recalibration, comparison with the metrological requirements for the intended use of the POVA, as well as any required sealing and labelling.

Nominal volume: upper limit of the usable volume range as specified by the manufacturer.

NOTES:
For a variable-volume piston-operated volumetric apparatus, the nominal volume corresponds to the maximum volume that can be set by the user and that is specified by the manufacturer.
For dispensers the nominal volume is depending on the tips used.

For the other terms refer to JCGM 200:2012 International vocabulary of metrology – Basic and general concepts and associated terms (VIM) [10] and ISO 8655-1 [3].

4. Best practices for the use of POVA and requirements for staff qualification

Guidance for the use of the different POVA, pipetting technique, as well as references to best practices for handling non-aqueous liquids are given in ISO 8655-10 [9]. Critical aspects to take into account, if applicable, include:

- Setting of the volume;
- Pre-wetting of pipettes tips/Immersion depth;
- Forward and reverse pipetting modes;
- Thermal equilibrium/Hand warming;
- Tip position during aspiration and dispense/Pause after aspiration;
- Velocity of aspiration and delivery;
- Tip wiping/Cleaning/Priming.

The ability to consistently deliver precise and accurate volumes is the volumetric performance, it is directly dependent on the system, the POVA and the technique employed by the user of the pipette. When using pipettes, the user's pipetting technique is usually the largest contribution to volumetric error. Therefore, operators who use, test or calibrate POVA shall be trained on liquid handling tool selection and proper pipetting techniques.

The laboratory shall establish and document the competence requirements for POVA users, including qualification and training requirements.

User competence can be monitored, e.g. by routine testing, proficiency testing or as inter-comparison between users.

A report of training or competence assessment can be issued to the user, whose content should include:

- Identification of the user
- Indication whether it is a training (trainer's identification) or competence assessment report
- Identification of the POVA and tips used
- Environment test conditions (at least room temperature and air relative humidity – RH)
- Volumes tested
- Criteria of the operator competence assessment (e.g. systematic and random errors or uncertainties in use of a single delivered volume for the tested volumes)
- Reference to ISO 8655-10 [9] (and any deviations) as well as the employed test method (e.g. ISO 8655-6 [6], ISO 8655-8 [8] or any specific test procedure in ISO 8655-7 [7])
- Volumetric measurement results for each delivered volume
- Total number of replicate measurements made for each tested volume, and the number of measurement results used for statistical analysis
- Date of test and duration of validity

5. General Consideration for LEVEL I on selection of POVA (including exchangeable parts)

All POVA shall be selected based on their suitability for the intended use. To achieve the best volumetric performance, it is recommended to select a POVA with a nominal volume close to the volume to be delivered. The following factors should be considered when selecting apparatus:

- smallest and largest liquid volume to be delivered;
- liquid properties;
- the resulting impact of delivering inaccurate volumes;
- performance requirements (maximum permissible errors – MPEs - and/or process tolerances);
- type and size of POVA and its application;
- single-channel or multi-channel POVA;
- delivery of constant volumes during repeated steps;
- frequency of use.

NOTE:
Refer to ISO 8655-10, Table A.1, for the selection of pipettes.

Exchangeable parts, such as pipette tips, shall be designed to match the design of the POVA. Changes in material, size of tip orifice, taper (angle), dead air volume and retained liquid impacts the performance of the pipetting system. The overall system performance (POVA and exchangeable parts) shall be suitable for its intended purpose.

NOTE:
Refer to ISO 8655-10, Table A.2, for the selection of pipette tips.

6. General Consideration for LEVEL II - Release for use

Check if all requirements set up during the selection are met and all necessary requirements are covered and fulfilled in the provided certificate.

7. General Consideration for LEVEL III - Calibration

7.1 Preliminary checks before calibration

Correct functionality of POVA shall be checked before calibration, as follows:

- Function of piston: smooth and positive.
- Tip holder: no marks or distortions, no liquid residues.
- Leakage: no drop is formed at the tip after 10 seconds from withdrawing the nominal volume.
- No internal or external contamination by visual checking.

7.2 Metrological confirmation

Some piston pipettes require the use of exchangeable parts during typical use (e.g. disposable or reusable pipette tips), that are considered to be an integral part of the POVA under test.

The periodic calibration of POVA shall follow a pre-established calibration plan, within the framework of the Quality Management System of the laboratory.

Calibration of the POVA shall be performed:

- on receipt (unless already calibrated by the supplier);
- on a regular basis and at least once every 12 months during the use of the pipette;
- after any maintenance, repair or adjustment.

As described in ISO 8655-1 [3], the laboratory can consider performing more frequent and regular calibrations based on a risk assessment, depending on:

- the workload of the POVA and number of operators using the pipette;
- the type of liquids used;
- the MPEs selected, depending on the type of use and the accuracy required.

If calibration is carried out by a service provider, the laboratory shall verify and choose a provider that is accredited in accordance with ISO/IEC 17025 [2] to perform calibrations in accordance with the requirements of ISO 8655.
If the calibration is performed by internal laboratory operators, the appropriate procedure for calibration shall be selected. Using the gravimetric (ISO 8655-6 [6]) or photometric (ISO 8655-8 [8]) measurement procedures, the laboratory shall be equipped with suitable balances, in relation to the volume of POVA to be calibrated, or spectrophotometers (plus density and pH meters).
If the above cannot be fulfilled, the laboratory should consider sub-contracting calibration to an accredited service provider or proceed with alternative measurement procedures for determination of volume as described in ISO 8655-7 [7].
ISO 8655-7 includes five test methods and the corresponding test procedures, described in Annex A to Annex E [7]. These methods are suitable for various maximum nominal volumes of POVA (see Table 1) and should be appropriately selected by the user, taking into account that they apply to complete systems comprising the basic apparatus and tips.

For calibrations, no less than ten replicate measurements per selected volume shall be performed. Measurement procedures and test conditions shall be validated for their suitability by comparison to one of the reference measurement procedures described in ISO 8655-6 [6] or ISO 8655-8 [8].

Table 1 - Summary of ranges of volumes tested in methods described in ISO 8655-7 [7]

Alternative test methods		ISO 8655-7 Annex	Volumes tested	
1	Gravimetric method	A	0.5 µL to 2 000 mL*	
2	Dual-dye ratiometric photometric method	B	0.1 µL to 5 000 µL	
3	Single dye photometric method	C	2 µL to 200 µL	
4	Hybrid photometric/gravimetric method for multi-channel POVA	D	1 µL to 100 µL in 96-well plates	1 µL to 50 µL in 384-well plates
5	Titration method	E	≥ 500 µL	
*range of volumes based on the minimum requirements for balances (see Table 3)				

Test conditions, equipment and procedure impact the uncertainty of measurement.

In addition, calibration shall be carried out to reflect routine operation (e.g. operational range of use; tips used for the calibration of pipettes should preferably be the same type as those used in daily work – in the case of sterilisable tips, the sterilisation procedures shall not affect the metrological characteristics of the tips (ISO 8655-2 [4]). **The exception is pipetting mode, where calibration shall only be performed in forward mode; calibration in reverse mode is no longer acceptable. For routine testing, reverse mode is acceptable.**

If the laboratory is working with infectious materials, decontamination (e.g. disassembling) should be done before calibration, according to the instructions provided by the manufacturer.

7.3 Environmental conditions

Ambient conditions (i.e. air temperature, RH, atmospheric pressure and water temperature) may influence weighing of the dispensed volume and water density (Z-factor); therefore, the calibration results can be impacted. As a result, calibration must be performed under controlled environmental conditions (i.e. temperature, humidity and pressure) to minimise the evaporation of water, which is critical for pipettes with dispensing volumes lower than 50 µL. Apart from the geometry of the weighing vessel (gravimetric method), the test cycle time (time required to complete the weighing of one delivered volume) is important and shall be kept to a minimum. Environmental condition requirements are given in Table 2.

Air temperature and RH shall be within the specified limits for the test room for at least 2 h before starting the test (minimum equilibration time) and during the test itself.

Table 2 - Summary of environmental conditions requirements described in ISO 8655 parts 6 and 8 vs part 7

Environmental conditions	**Requirements**	
	ISO 8655-6, -8[*]	ISO 8655-7[*, **]
Test room temperature***	20 ± 3 °C	To be recorded
Temperature variation during test	0.5 °C	0.5 °C [*]/to be recorded [**]
Temperature variation during 2 h equilibration	0.5 °C / h	1 °C [*]/ to be recorded [**]
Water[*] / test liquid[**] temperature	± 0.5 °C of ambient air temperature	To be recorded
Test room humidity	45 - 80 %	To be recorded
Air pressure	To be recorded	To be recorded
[*] For calibrations		
[**] For verification/routine testing to be recorded (the test room conditions should reflect the environmental conditions under which the POVA is used.) ***When the POVA is required for use in a country which has adopted a standard reference temperature of 27 °C (the alternative temperature recommended in ISO 384 [1] for such use), this figure shall replace the reference to 20 °C.		

7.4 Gravimetric test

The principle of the gravimetric method is described in ISO 8655-6 [6]. If the selected volume is lower than 50 µL, the effect of the evaporation of the water used is significant. Therefore, the loss of mass should be taken into account in the calculation.

Settings:

- The laboratory where the calibration is performed shall be draught free, with RH of 45-80 %. If volumes lower than 50 µL are selected, an evaporation trap or an open vessel containing water shall be placed inside the balance chamber, and the test cycle time shall be kept to a minimum.
- In addition, the temperature of the laboratory shall be 20 ± 3 °C and stable within ± 0.5 °C during calibration.
- All the material needed for the test (glassware, water, exchangeable parts, etc.) must be equilibrated for at least 2 h in the room where the test is to be performed.
- If using a variable-volume POVA, select the test volume; this setting shall not be altered during the test cycle of all replicate measurements.

Equipment:

- An analytical balance with appropriate characteristics shall be used depending on the selected volume of the POVA under test (according to ISO 8655-6 [6]), as given in Table 3.

Table 3 - Minimum requirements for balances

Nominal volume of apparatus under test (V)	**Resolution** (d) mg	**Repeatability** (s)[a] mg	**Expanded uncertainty in use** U (k = 2)[a, b] mg
0.5 µL ≤ V < 20 µL	0.001[c] 0.01[d]	0.006[c, e] 0.03[d]	0.012[c, e] 0.06[d]
20 µL ≤ V < 200 µL	0.01	0.025	0.05
200 µL ≤ V ≤ 10 mL	0.1	0.2	0.4
10 mL < V ≤ 1 000 mL	1	2	4
1 000 mL < V ≤ 2 000 mL	10	10	40

[a] The repeatability and expanded uncertainty in use value, in this table, apply in the volume determination of a single-channel apparatus. When a single-channel balance is used exclusively for volume determination of multi-channel pipettes, the repeatability and expanded uncertainty in use values are double the values of this table. See also Footnote d.
[b] Expanded uncertainty in use can be estimated according to EURAMET cg-18 Version 4.0 [11] or ASTM E898 [12], at the value of the nominal volume. Expanded uncertainty in use may be taken from the balance calibration certificate or calculated separately (see example in ISO/TR 20461 [13]).
c Single-channel balance.
[d] Multi-channel balance, only valid for multi-channel pipettes. Multi-channel balances of 0.01 mg readability may be used to test multi-channel pipettes with nominal volumes below 20 µL only if the expanded uncertainty in use is less than one-quarter of the maximum permissible systematic error of the apparatus.
[e] For single-channel pipettes of nominal volumes of less than 2 µL, a balance with repeatability and an expanded uncertainty better than the values in the table shall be used so that the expanded uncertainty in use is less than one-quarter of the maximum permissible systematic error of the apparatus.

- Different measurement devices are needed for environmental condition monitoring and shall have, according to ISO 8655-6 [6], the minimum requirements listed in Table 4.

Table 4 - Minimum requirements for measuring devices

Device	**Resolution**	**Expanded uncertainty of measurement** (k = 2)
Thermometer for liquids	0.1 °C	0.2 °C
Thermometer for room air	0.1 °C	0.3 °C
Hygrometer	1 % relative humidity	5 % relative humidity
Barometer	0.1 kPa	1 kPa
Timing device	1 s	not applicable

- The weighing vessel shall be clean and have enough liquid inside to cover the bottom of the vessel when the measurement procedure is started, to keep the RH sufficiently high.
- The weighing vessel should be of suitable size (height/diameter ratio of at least 3:1), filled with purified water to a depth of at least 3 mm.
- A reservoir vessel should be used, containing purified water (minimum quality level 3, see standard ISO 3696 [14]) to an excess that allows the performance of all tests, e.g. greater than ten times the dispensed volume.
- Tips used should be appropriate for the POVA to be calibrated.

Method:

1. Test room

- **At the start and at the end** of the measurements, the temperature of the test liquid shall be recorded. The air temperature, the barometric pressure and the RH in the test room shall be recorded.

NOTE:
Air temperature and barometric pressure are necessary for the calculation of the correction factor Z; the relative humidity is necessary for the stability of the room conditions. All should be documented in the test report.

2. Selection of volumes:

- In the case of a **fixed-volume** apparatus, the test volume is the nominal volume.
- Variable-volume pipettes (single- or multi-channel) shall be tested at three different volumes, each with 10 replicates:
    - the nominal volume;
    - 50 % of the nominal volume;
    - the lower limit of the useful volume range or 10 % of the nominal volume (whichever is greater).
- Each channel of **multi-channel** pipettes shall be considered as a single channel and tested and reported as such. All channels of a multi-channel pipette should be tested individually to account for its specific design and operation.

  Multi-channel balances can be used to measure the test volume delivered from all channels in parallel, by aspirating and emptying each channel at the same time and evaluating the results of each channel individually (if the pipette has more than one row of channels, it may be tested one row at a time).

  Each channel can be measured individually, one after another, with a single-channel balance. In these conditions, test liquid shall be aspirated by all channels together and collected from one channel at a time, if feasible. For the measurement of channel 1, for example, the volume of channel 1 is delivered into the weighing vessel (e.g. a narrow tube), while the volumes from all other channels are discarded (e.g. into a petri dish).
- **Dispensers** (as repetitive piston pipettes/multi delivery dispensers) should be calibrated with the tips routinely used, taking into account the different moulds and fittings. The calibration shall be carried out by selecting the lowest (lower limit of the useful volume range or 10% of the nominal volume, whichever is greater), intermediate (50% of the nominal volume) and the highest dispensed volume of the aliquots. For each selected volume 10 replicates should be made, for a total of 12 dispensing steps considering that the first and the last volume dispensed should be discarded as they are considered to be an excess and a residual, respectively, and not equivalent to the set volume.
- For **electronic motorised pipettes** where aspiration and delivery of test liquid are automatic, forward pipetting shall always be performed. The calibration should be performed in pipetting mode unless testing specific requirements (e.g. multi-dispensing mode may be performed in addition, see note below) and should refer to the manufacturer's instructions or operation manual for speed settings of aspiration and delivery.

NOTE:
In multi-dispensing mode the verification is based on the nominal volume, by performing 10 measurements that represent 10 % of the nominal volume of the pipette (e.g. for an electronic motorised pipette with a nominal volume of 200 µL, 10 measurements of 20 µL are performed).

3. Place the appropriate tip on the pipette.
4. For variable-volume pipettes select the volume to be tested[1].
5. Depress plunger.
6. For air displacement pipettes, fill the pipette at least five times with the test liquid and discard the fillings (pre-wetting to reach humidity equilibrium).
7. Hold the pipette vertically (not touching the side walls or bottom of the vessel), immerse the tip in the test liquid to the appropriate depth below the surface of the test liquid (insufficient immersion can result in the aspiration of air, very deep immersion can influence the volume of liquid aspirated due to variability of the hydrostatic pressure as a function of immersion depth and increases the chance of droplets clinging to the exterior of the tip - see Table 5).

Table 5 - Immersion depths during aspiration and wait time after aspiration of test liquid

Volume [µL]	**Immersion depth** [mm]	**Wait time** [s]
≤ 1	1 to 2	1
> 1 to 100	2 to 3	1
> 100 to 1 000	2 to 4	1
> 1 000 to 20 000	3 to 6	3

8. Liquid aspiration into the pipette tip should be done with slow speed, pushing the plunger smoothly until coming to rest with a light and consistent force at the first stop, then releasing the plunger at a constant rate and pause for 1-3* s (*for volumes higher then 1 000 µL).
9. Pull the pipette tip slowly out of the liquid, vertically (pipette tip should be inspected to ensure that no sample liquid droplets are clinging to the outside of the tip).
10. Tare the balance to zero ($m_0 = 0$)
11. If the weighing vessel has a lid, remove it.
12. Depress the plunger and deliver the test liquid slowly into the weighing vessel.
13. When delivering the test liquid into the weighing vessel, touch with the tip against the recipient wall near the liquid surface, holding the pipette at an angle of 30-45 °, sliding the tip along the wall over 8-10 mm before releasing the plunger (to ensure no droplets adhere to the tip when removed from the vessel).
14. Close the lid of the weighing vessel, if appropriate.
15. Record the balance indication m_i (i = 1 to n) of the weighing vessel with the delivered test liquid (if tare is used m_i will be the delivered quantity of water) and calculate the weighing value as the difference between the balance indications.
16. Repeat the test cycle (withdrawing and dispensing of the test liquid), starting at step 5, until all measurements have been performed and values recorded.

NOTES:

1. Pipettes with screw-type plunger mechanisms should be turned at least one-third of a revolution (where possible) above the desired volume and then turned down to the desired volume. This ensures that the micro bolt gears align in the same configuration every time a volume is set. Adjustable-volume POVA should be returned to the nominal volume setting for storage.
2. Hand-warming of the pipette by using it for extended periods of time (longer than 10 minutes) should be avoided. Heat transfer from the hand can lead to a thermal disequilibrium and affect the volume of the air cushion of the pipette and its mechanical parts, introducing volumetric errors (this can be mitigated wearing gloves and routinely returning pipettes to its stand).
3. For multiple delivery dispensers (see ISO 8655-5), do not reset the piston between each of the *n* test cycles to its initial position if there is sufficient test liquid remaining to deliver the next aliquot.
4. During the replicate measurements, the pipette tip shall be changed at least once in order to detect the use of damaged or incorrectly manufactured tips and assess the variability of the used tips. For n = 10 replicates, at least two tips shall be used, and the tip shall be changed at minimum once every 5 measurements. When replacing the tips, they shall be pre-wetted, starting the test cycle at step 5). This tip change is also applicable to positive displacement pipettes with disposable tips (type D2), but not applicable to dispensers.
5. During the test cycle of electronic motorised pipettes in multi-dispensing mode, it should be taken into account that:
   - Depending on predefined recommendations for the setup of the pipettes, it can be necessary to first dispense a discard volume after the test liquid has been aspirated (refer to the manufacturer's instructions).
   - After the motor completed the first dispense, pause to allow complete dispensing (liquid stops moving). While removing the pipette, draw the tip along the inside surface of the receiving vessel to ensure that the last drop stays inside the receiving vessel.
   - Repeat the operation for the second to tenth aliquots without changing the tip, or aspirating test liquid, as long as enough liquid for 10 replicates has been aspirated. When the amount of test liquid that can be aspirated is not enough for 10 replicates, the tip should be changed before aspirating the next amount of test liquid. In this case, pre-rinsing is required once again.
   - Discard the residual liquid.
6. If it is necessary to remove the weighing vessel from the balance pan to enable delivery of the volume, avoid excessive handling and possible contamination by the use of lint-free gloves (this action will increase the measurement uncertainty of the procedure).
7. If a mathematical compensation of evaporation is performed, note the time to the nearest second taken to complete the 10 test cycles. Perform one or two evaporation trials mimetising one dispense, but without delivering liquid. Follow the same methodology as an actual delivery. Record the mass of the weighing vessel before and after each evaporation trial (see Formula 1).

Calculations:

a) Evaporation loss can be determined by calculating the difference between the starting mass (m_{00}) and the mass after the first cycle time without making any delivery of liquid (m_0). This can be repeated again at the end of the test, to obtain m_n and m_{n+1}.

The average evaporation loss per test cycle can be determined using either Formula 1 or another appropriate method or formula.

$$m_{evap} = \frac{(m_{00} - m_0) + (m_n - m_{n+1})}{2} \qquad (1)$$

where

m_{evap}	is the estimated evaporated mass within a test cycle;
m_{00}	is the balance indication at the first reading (starting mass);
m_0	is the balance indication after the cycle time;
m_n	is the balance indication after the *n*th replicate delivered;
m_{n+1}	is the balance indication after the *n*th replicate delivered after the cycle time.

b) Calculate the mean temperature *t* of the test liquid (rounded to the nearest 0.5 °C).

$$t = \frac{t_1 + t_2}{2} \qquad (2)$$

t mean temperature
t_1 temperature before the first weighing
t_2 temperature after the last weighing

c) Use the barometric pressure B and mean temperature *t* to find the corresponding Z-factor from Table 6, below.

d) Calculate the volume V_i in µL from the individual masses m_i.

$$V_i = Z \times (m_i + m_{evap}) \quad (3)$$

V_i individual volume (calculated)
m_i weight of the individual volume
m_{evap} evaporation loss

e) Calculate the mean volume $\overline{V}$ from the series of volumes.

$$\overline{V} = \frac{\sum V_i}{n} \qquad (4)$$

$\overline{V}$ mean of the individual volumes from the series
V_i individual volume (calculated)
n number of weighings in the series

f) Calculate the systematic error (trueness) *e*, which is the difference between the mean volume of actual measurements and the true value as specified by the selected volume V_s. Trueness is expressed in µL.

$$e = \overline{V} - V_s \qquad (5)$$

e accuracy
$\overline{V}$ mean of the individual volumes from the series
V_s selected volume

g) Systematic error can be also expressed as a percentage.

$$e_s = 100\% \times (\bar{V} - V_s)/V_s \qquad (6)$$

e_s is the relative systematic error of measurement

h) Calculate the random error (repeatability or standard deviation) *s*, which quantifies the scattering of individual weighing of dispensed volumes as an absolute value (µL). Random error can be also expressed as a percentage by the coefficient of variation (CV). Formulas are provided below:

$$s = \sqrt{\frac{\sum (V_i - \overline{V})^2}{n-1}} \qquad (7)$$

s repeatability, standard deviation
Vi individual volume (calculated)
$\overline{V}$ mean of the individual volumes from the series
n number of weighing in the series

$$CV = \frac{100 \times s}{\overline{V}} \qquad (8)$$

CV coefficient of variation

Table 6 - Z-correction factors for distilled water as a function of water temperature and air pressure

Z values in microlitres per milligram

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

8. General Consideration for LEVEL IV - Routine tests

8.1 Preliminary checks before routine testing

Before routine use, traceability and labelling (e.g. unique identification code, valid calibration date) of the pipettes shall be checked.

Preliminary checks such as functional testing also apply to verification.

8.2 Metrological confirmation

The routine testing of the POVA shall be performed at regular intervals as part of test equipment monitoring or analytical quality control routines (e.g. prior to use, every three months). The frequency adopted can be chosen based on a risk analysis, considering the following factors:

- risk of application;
- workload of use;
- number of users of the POVA;
- aggressive nature of the liquid to be delivered and its vapours;
- acceptable MPEs;
- manufacturer's information;
- number of dispenses performed on each occasion of use;
- liquid handling process requirements.

Verification can be performed following the procedures specified in ISO 8655-6 [6] and ISO 8655-8 [8].

Alternative measurement procedures and requirements, as described in ISO 8655-7 [7], are suitable to be used in the metrological confirmation of POVA and allow deviations from the strict requirements of the reference measurement procedure (ISO 8655-6 [6] or ISO 8655-8 [8]).
To assess the correct functioning of POVA, the test room conditions should reflect the environmental conditions for routine use. Deviations from reference method requirements are described in Table 2.

All test equipment used should be of suitable readability, accuracy and reproducibility.
The test liquids and receiving vessels can differ (e.g. cuvettes) depending on the selected method. Verification may be performed using liquids or solutions other than water.

NOTES:

The test liquid preparation shall be described in sufficient detail to allow replication of the test and interpretation of the results. If it is to be stored for any length of time, its stability should be known.

Several characteristics of the test liquid shall be taken into account when determining the measured volume: *Z*-factor when weighing, absorbance of the chromophore when using photometric methods, and conductivity and reactivity when performing potentiometric titration.

Depending on the type of POVA, different parameters can influence the volume of liquid aspirated and/or dispensed: viscosity, density, chemical composition and surface tension. The influence of the test liquid on the expanded uncertainty of measurement shall be accounted, if needed.

Deviations from reference method requirements for test conditions are also permitted and are described in Table 7.

Table 7 - Summary of test condition requirements described in ISO 8655 parts 6 and 8 vs part 7

Test conditions (as left measurement)	**Requirements**	
	ISO 8655-6, -8	ISO 8655-7
Calibration / Test points	≥ 3*	≥ 3*/ ≥ 1**, ***
Calibrated / Tested volumes	Nominal volume, 50 %, 10 % or min if min > 10 %	Nominal volume, 50 %, 10 % or min if min > 10 %
Measurements per point (n)	≥ 10*	≥ 10* or ≥ 4** (if the expanded uncertainty of measurement for the POVA is fit for the intended purpose)
Tip change (at n/2)	At least one tip change per measured volume	At least one tip change per measured volume*/ Not defined**
* For calibrations		
** For routine testing, fewer than three volumes may be tested. In case the POVA is to be tested at only two volumes, the nominal volume and the lower limit of the usable volume range, or 10 % of the nominal volume (whichever is the greater), shall be tested.		
*** For routine testing, in case the POVA is tested only at one volume, it shall be tested at its nominal volume, or at the volume at which it will be used.		

9. POVA Evaluation

9.1 Determination of pass/fail status

Appropriate performance tolerances for POVA, suitable for the purpose of its intended use, shall be defined.

The pass/fail status shall be evaluated by comparing the determined systematic and random errors against the maximum permissible systematic and random errors defined by:

1. POVA product tolerances, for example those provided in ISO 8655-2 [4] (Tables 8 to 10) for pipettes; or
2. POVA product tolerances, for example those provided in ISO 8655-5 [5] (Tables 11 and 12) for dispensers; or
3. POVA product tolerances provided by the POVA manufacturer; or
4. other values established by the POVA user.

9.2 Non-conformity of pipettes

If a metrological confirmation gives non-compliant results, the pipettes should be taken out of service until adjustment and successful calibration/verification have taken place.

9.3 Calibration/report

After the calibration, the results shall be appropriately reported according to ISO/IEC 17025 [2] requirements.
The calibration report may be issued as hard copy or by electronic means, containing the following information:

1. Title of the report/certificate.
2. Identification of the report/certificate.
3. Entity responsible for the calibration.

4. Identification of the POVA (or internal code):
   - manufacturer's name;
   - type name or model number;
   - manufacturer's serial number or organisation's unique asset tag number;
   - nominal volume of the POVA or usable volume range.
5. Identification of the reference document followed, i.e. ISO 8655-6 [6], -7 [7], -8 [8].
6. Any variation or deviation from the reference measurement.
7. Reference to the alternative procedure used: reference to ISO 8655-7 [7], as well as the employed test method and procedure (e.g. "ISO 8655-7:2022, D" if the photometric/gravimetric hybrid procedure according to Annex D was performed).
8. Basis of the test: measurement by delivery (Ex) or contained (In).
9. Conditions of measurement (water temperature[1]; room conditions: temperature, pressure and humidity).
10. Z-factor (for the gravimetric method).
11. Unique identification of the equipment used to perform the calibration (balance, hygrometer, thermometer, etc.) unless specified in another quality document.
12. Identification of the tips and other exchangeable parts used with the POVA for the test, e.g. brand, model and serial or lot number (calibration).
13. Type of test liquid (ISO 8655-7 [7]).
14. Reference to the formula used to convert weighing values into volume.
15. Calibration results for each delivered volume (where applicable, before and after maintenance).
16. Total number of replicate measurements made for each selected volume, and the number of measurement results used for statistical analysis (calibration).
17. Expanded uncertainty of the mean delivered volume, for each selected volume and channel (if required, e.g. calibration).
18. Calculation of systematic and random measurement error obtained for the selected volumes.
19. Acceptance criteria (if applicable).
20. Conclusion(s), if applicable[2].
21. Identification of the operator performing the test and the person authorising the report.
22. Date of calibration.
23. Page numbering.

NOTES:

[1] Cubic thermal expansion coefficient γ, if a correction for cubic thermal expansion of the POVA is made.

For electronic motorised pipettes the results report should indicate the operation mode (pipetting and, if applicable, multi-dispensing mode) and the aspiration and dispense speed settings (if available by the manufacturer).

[2] If the external provider is accredited to evaluate the results through provision of the MPEs, the customer may request the external provider to issue a conclusive report.

For routine testing a report is not required, although conditions and results shall be documented.

9.4 Requirements and related typical acceptance criteria

Uncertainty of measurement:

For calibrations (ISO 8655-1 [3]), the expanded measurement uncertainty of the mean delivered volume for each selected volume shall be estimated and reported. It shall include contributions from the measurement procedure and the device under test.

Limits:

The MPEs always apply to the total system of POVA and tip.

In order to state the metrological trueness and precision of the POVA and thus determine its systematic and random errors, a reference measurement procedure as specified in ISO 8655-6 [6] and ISO 8655-8 [8], or another method in accordance with ISO 8655-7 [7], shall be used. The MPEs given in Tables 8 to 10 (single-channel and multi-channel pipettes) as well as in Tables 11 and 12 (dispensers), or other applicable specifications, shall be applied.

Alternatively, liquid handling process tolerances can be used as performance tolerances; regional, national, industry-specific or organisation-specific requirements for performance tolerances can apply. Furthermore, the laboratory may specify other limits in order to meet its requirements or manufacturer's specifications. For the definition of these differing limits, the uncertainty of measurement should be taken into consideration. In this case, the limits defined must be justified and must take into account the use of each POVA.

The calculation of maximum permissible systematic and random error in the usable volume range, not included in Tables 8 to 12, shall be made by dividing the nominal volume by the selected volume and multiplying the result by the MPEs at nominal volume (Formula 9). This calculation does not apply to volumes below 10 % of the nominal volume.

$$e_{Vs} = \frac{V_{nom}}{V_s} \text{ x } e_{Vnom} \qquad (9)$$

where

V_{nom}	is the nominal volume;
V_s	is the selected volume;
e_{Vnom}	is the MPE (either systematic or random) at nominal volume;
e_{Vs}	is the MPE (either systematic or random) at the selected volume.

If the calculated value exceeds 25 %, then the value of 25 % shall be applied as the maximum permissible error.

EXAMPLE: Single-stroke dispenser with a nominal volume of 10 mL and a usable volume range of 1–10 mL.

Calculation of maximum permissible systematic error at a selected volume of 2 mL (Table 11):

$e_{Vnom} = 0.60$ %

$V_{nom} = 10$ ml

$V_s = 2$ ml

$$e_{Vs} = \frac{V_{nom}}{V_s} \text{ x } e_{Vnom}$$

$$e_{Vs(2\ ml)} = \frac{10\ ml}{2\ ml} \text{ x } 0.6\ \%$$

$$e_{Vs(2\ ml)} = 5 \text{ x } 0.6\ \% = 3\ \%$$

In the case of electronic motorised pipettes, the following specifications shall be applied:

1. For single-channel pipettes, the results may be compared with Table 8, or other applicable specifications.
2. For multi-channel pipettes, the results may be compared with Table 9, or other applicable specifications.

NOTES
In multi-dispensing mode the nominal volume remains that of the pipetting mode and the specifications of the pipetting mode nominal volume apply.

For routine testing, the performance tolerances (limits) shall be defined and documented. Performance tolerances may be based on:

- the user's liquid handling process tolerances;
- the product tolerances given in the part of ISO 8655 corresponding to the type of POVA under test;
- the tolerances specified by the manufacturer, if fit for purpose;
- other tolerances, in order to meet the laboratory's requirements.

The following approaches are suggested as a starting point for developing achievable tolerance limits:

1. Doubling the limits stated in ISO 8655 (Tables 8-12).
2. Two percent of full scale at all volume settings, applicable up to 20 µL [15].
3. Considering the requirements of the analytical method in which the pipette is used, in terms of permitted errors for measuring sample or standard volume, depending on the test (chemical, biological or microbiological).
4. If historical data are available, a statistical approach (e.g. control charts) can be used.

Setting performance tolerances that are too narrow can result in unnecessary calibration and routine test failures, even though the liquid handling process tolerances can accommodate a wider range of delivered volumes. Settings tolerances that are too wide can prevent the detection of damage to the POVA.

Table 8 - MPEs for types A and D1 (single-channel pipettes)

Pipetting volume		**Maximum permissible systematic error[a] ±%**	**Maximum permissible random error[a] %[b]**
Nominal volumes µL	**Setting as a proportion of the nominal volume %**		
1 to 3[c]	100	2.5	2.0
	50	5.0	4.0
	10	25	20
> 3 to 5	100	2.5	1.5
	50	5.0	3.0
	10	25	15
> 5 to 10	100	1.2	0.8
	50	2.4	1.6
	10	12	8.0
> 10 to 50	100	1.0	0.5
	50	2.0	1.0
	10	10	5.0
> 50 to 5 000	100	0.80	0.30
	50	1.6	0.60
	10	8.0	3.0
> 5 000 to 20 000	100	0.60	0.30
	50	1.2	0.60
	10	6.0	3.0

a To calculate errors in microlitres, multiply the MPEs by the selected volume.

b Expressed as the coefficient of variation according to ISO 8655-6 [6], ISO 8655-7 [7] or ISO 8655-8 [8].

c Handling of such low volumes can be very challenging.

Table 9 - MPEs for types A and D1 (multi-channel pipettes)

Pipetting volume		**Maximum permissible systematic error[a] ±%**	**Maximum permissible random error[a] %[b]**
Nominal volumes µL	**Setting as a proportion of the nominal volume %**		
2[c]	100	8.0	8.0
	50	16	16
	10	25	25
> 2 to 5	100	5.0	3.0
	50	10	6.0
	10	25	25
> 5 to 10	100	2.4	1.6
	50	4.8	3.2
	10	24	16
> 10 to 20	100	2.0	1.0
	50	4.0	2.0
	10	20	10
> 20 to 50	100	2.0	0.80
	50	4.0	1.6
	10	20	8.0
> 50 to 2 000	100	1.6	0.60
	50	3.2	1.2
	10	16	6.0

a To calculate errors in microlitres, multiply the MPEs by the selected volume.

b Expressed as the coefficient of variation according to ISO 8655-6 [6], ISO 8655-7 [7] or ISO 8655-8 [8].

c Handling of such low volumes can be very challenging.

Table 10 - MPEs for type D2

Pipetting volume		**Maximum permissible systematic error[a] ±%**	**Maximum permissible random error[a] %[b]**
Nominal volumes µL	**Setting as a proportion of the nominal volume %**		
5[c]	100	2.5	1.5
	50	5.0	3.0
	10	25	15
> 5 to 10	100	2.0	1.0
	50	4.0	2.0
	10	20	10
> 10 to 20	100	2.0	0.80
	50	4.0	1.6
	10	20	8.0
> 20 to 100	100	1.4	0.60
	50	2.8	1.2
	10	14	6.0
> 100 to 1 000	100	1.2	0.40
	50	2.4	0.80
	10	12	4.0

a To calculate errors in microlitres, multiply the MPEs by the selected volume.

b Expressed as the coefficient of variation according to ISO 8655-6 [6], ISO 8655-7 [7] or ISO 8655-8 [8].

c Handling of such low volumes can be very challenging.

Table 11 - MPEs for single-stroke dispensers

Dispensing volume		**Maximum permissible systematic error[a] ±%**	**Maximum permissible random error[a] %[b]**
Nominal volumes mL	**Setting as a proportion of the nominal volume %**		
0.01	100	2.0	1.0
	50	4.0	2.0
	10	20	10
> 0.01 to 0.02	100	2.0	0.50
	50	4.0	1.0
	10	20	5.0
> 0.02 to 0.05	100	1.5	0.40
	50	3.0	0.80
	10	15	4.0
> 0.05 to 0.1	100	1.5	0.30
	50	3.0	0.60
	10	15	3.0
> 0.1 to 0.2	100	1.0	0.30
	50	2.0	0.60
	10	10	3.0
> 0.2 to 0.5	100	1.0	0.20
	50	2.0	0.40
	10	10	2.0
> 0.5 to 200	100	0.60	0.20
	50	1.2	0.40
	10	6.0	2.0

[a] To calculate errors in millilitres, multiply the MPEs by the selected volume.

[b] Expressed as the coefficient of variation according to ISO 8655-6 [6], ISO 8655-7 [7] or ISO 8655-8 [8].

Table 12 - MPEs for multiple delivery dispensers

Dispensing volume		**Maximum permissible systematic error[a]**	**Maximum permissible random error[a]**
Nominal volumes mL	**Setting as a proportion of the nominal volume** %	**±%**	**%[b]**
0.001 to 0.002	100	5.0	5.0
	50	10	10
	10	25	25
> 0.002 to 0.003	100	2.5	3.5
	50	5.0	7.0
	10	25	25
> 0.003 to 0.01	100	2.0	2.5
	50	4.0	5.0
	10	20	25
> 0.01 to 0.02	100	1.5	2.0
	50	3.0	4.0
	10	15	20
> 0.02 to 0.05	100	1.0	1.5
	50	2.0	3.0
	10	10	15
> 0.05 to 0.2	100	1.0	1.0
	50	2.0	2.0
	10	10	10
> 0.2 – 0.5	100	1.0	0.60
	50	2.0	1.2
	10	10	6.0
> 0.5 to 1	100	1.0	0.40
	50	2.0	0.80
	10	10	4.0
> 1 to 2	100	0.80	0.40
	50	1.6	0.80
	10	8.0	4.0
> 2 to 5	100	0.60	0.30
	50	1.2	0.60
	10	6.0	3.0
> 5 to 25	100	0.50	0.30
	50	1.0	0.60
	10	5.0	3.0
> 25 to 200	100	0.50	0.25
	50	1.0	0.50
	10	5.0	2.5

a To calculate errors in millilitres, multiply the MPEs by the selected volume.

b Expressed as the coefficient of variation according to ISO 8655-6 [6], ISO 8655-7 [7] or ISO 8655-8 [8].

10. References

(For all references, if the version is not mentioned, the latest version applies)

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