International Standard

ISO 8655-10

First edition
2024-02

Piston-operated volumetric apparatus —

Part 10: User guidance, and requirements for competence, training, and POVA suitability

Appareils volumétriques à piston —

Partie 10: Recommandations d'utilisation et exigences relatives aux compétences et à la formation des utilisateurs, ainsi qu'à l'adéquation des AVAP

Reference number
ISO 8655-10:2024(en)

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Published in Switzerland

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 48, *Laboratory equipment*.

A list of all parts in the ISO 8655 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document addresses the needs of piston-operated volumetric apparatus (POVA) users and quality and laboratory managers, and serves as a basis for:

— user guidance, training, and qualification;

— establishing POVA performance and test requirements to ensure fitness for their intended use;

— selecting pipetting equipment.

The tests specified in the ISO 8655 series are intended to be carried out by trained personnel.

Piston-operated volumetric apparatus —

Part 10:
User guidance, and requirements for competence, training, and POVA suitability

1 Scope

This document provides user guidance regarding the selection of piston-operated volumetric apparatus (POVA) (including exchangeable parts) and best practices for their use.

This document also specifies requirements for user training and competence. Further, this document introduces performance tolerances and testing of POVA to ensure fitness for their intended use.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 8655-1, *Piston-operated volumetric apparatus — Part 1: Terminology, general requirements and user recommendations*

ISO/IEC Guide 2, *Standardization and related activities — General vocabulary*

ISO/IEC Guide 99, *International vocabulary of metrology — Basic and general concepts and associated terms (VIM)*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 8655-1, ISO/IEC Guide 2, and ISO/IEC Guide 99 apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Requirements and best practices

4.1 Selection of POVA

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;

— its application;

— the resulting impact of delivering inaccurate volumes;

— performance requirements (maximum permissible errors and/or process tolerances);

— type and size of POVA equipment;

— single-channel or multi-channel POVA;

— delivery of constant volumes during repeated steps;

— frequency of use.

NOTE Refer to Table A.1 for the selection of pipettes.

4.2 Selection of exchangeable parts

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 impact the performance of the pipetting system.

The overall system performance (POVA and exchangeable parts) shall be suitable for its intended purpose.

NOTE Refer to A.2 for the selection of pipette tips.

Tips made of plastic for air-displacement pipettes are designed for single use. They shall not be cleaned for reuse as their metrological characteristics will no longer be reliable.

Single use of a pipette tip means mounting the tip to the pipette only once and then discarding it after use. While the tip is mounted to the pipette, it may be used to handle several replicate aspiration and delivery cycles, as long as a tight seal between the tip and pipette's tip cone is maintained, and no risk for cross-contamination exists.

4.3 Best practices

4.3.1 General

Reference shall be made to the user's manual for the POVA.

The performance of many laboratory instruments, including POVA, is subject to the user's technique (operator effect). When using pipettes, the user's pipetting technique is usually the largest contribution to volumetric error.[1] Guidance for the use of air-displacement pipettes is given in 4.3.3, and for the use of positive displacement pipettes in 4.3.5. Guidance for the use of burettes, dilutors, dispensers, and syringes is described in 4.3.6, 4.3.7, 4.3.8, and 4.3.9 respectively.

When handling non-aqueous liquids such as viscous, volatile, biological, surfactant-added, or corrosive liquids, it is generally recommended to use positive displacement devices. When using air-displacement pipettes for these liquids, reverse pipetting technique should be applied.

NOTE ISO 8655-2:2022, Annex A, identifies and quantifies possible sources of error for air-displacement pipettes. The report of EURAMET project no. 1295[2] also quantifies common errors when using air-displacement pipettes.

4.3.2 Setting of the volume (variable volume POVA)

Setting the volume of the POVA to the desired volume is critical for the trueness of the delivered volumes. 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. Dialling down 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.

4.3.3 Air-displacement pipettes

4.3.3.1 Pipetting technique

Small variations in a user's pipetting technique can lead to significant errors in the delivered volume. The errors quantified in ISO 8655-2:2022, Annex A, and EURAMET project no. 1295[2] can be additive and result in cumulative volumetric errors that can have a substantial impact on laboratory results. Using proper pipetting technique, described in 4.3.3.2 to 4.3.4.6, minimizes errors and ensures consistently reliable delivered volumes.

The direct dependency of reliable pipetting results on the employed pipetting technique makes it imperative to train users of handheld pipettes on the proper use of such devices and assess each user's pipetting skills on a regular basis (see Reference [3] for more information).

4.3.3.2 Pre-wetting of pipette tips

After fitting a new tip to the pipette, the desired volume of sample solution should be aspirated and dispensed at least five times (more in environments of very low humidity). This process reduces losses due to sample retention and increases the partial vapour pressure in the air cushion, reducing errors from sample evaporation in the pipette tip. Care should be exercised not to aspirate air into the pipette tip between the dispense and next aspiration of the sample.

When pipetting liquids with high vapour pressure, e.g., many organic solvents, more than five pre-wetting cycles should be completed.

4.3.3.3 Forward and reverse pipetting modes

Aqueous liquids are typically pipetted in forward mode as this is the mode in which pipettes are typically calibrated. Reverse pipetting can result in a bias that shall be considered. Generally, forward mode pipetting of aqueous liquids results in better accuracy.

NOTE Some air-displacement pipettes can have the following piston positions:

— neutral position, where the piston is not depressed;

— first stop, where the piston is depressed to the first stop;

— second stop (sometimes called blow-out mode), where the piston is depressed beyond the first stop.

When pipetting solutions with aqueous properties, forward mode pipetting should be practiced. The plunger should be depressed to the first stop, then the pipette tip is immersed into the sample solution, and subsequently, the plunger should be released at a slow yet steady rate to aspirate the sample. The sample should be dispensed into the destination receptacle, preferably against its side wall, by depressing the plunger to the first stop, pausing if possible, and then depressing it to the second stop.

Some liquids, e.g. viscous liquids, may advocate the use of reverse mode pipetting. The plunger should be depressed to the second stop, then the pipette tip is immersed into the sample liquid, which should be aspirated at a slow yet steady rate. The sample liquid should be dispensed into the destination receptacle by depressing the plunger to the first stop only. A portion of aspirated sample liquid should remain in the pipette tip after the desired amount has been dispensed. When using filter tips in reverse mode, care should be used that the aspirated liquid does not come into contact with the filter.

4.3.3.4 Thermal equilibrium

The air cushion between the plunger and the sample solution is susceptible to temperature influences (see Reference [4] for more information). The pipette, pipette tip, and sample liquid should therefore be in thermal equilibrium for correct volumetric results. In cases when thermal equilibrium cannot be established (warm or cold liquids), the effect on the accuracy of the delivered volume shall be considered, or positive displacement pipettes should be used (see 4.3.5).

4.3.3.5 Hand warming

Hand-warming of the pipette by using it for extended periods of time 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. Transmission of warmth from the hand to the pipette can be mitigated by wearing gloves, avoiding continuous use of pipettes for extended periods of time (longer than 10 minutes), routinely returning pipettes to the designated pipette stand, and by selecting pipette models, which are designed to minimize heat transfer from the user's hands.

4.3.3.6 Immersion depth

The orifice of the pipette tip should be immersed to the appropriate depth below the surface of the sample liquid and remain at this depth while aspirating sample liquid into the pipette tip. Immersing the pipette tip not deeply enough below the surface of the sample liquid can result in the aspiration of air. Immersing the tip too deeply below the surface can influence the volume of liquid aspirated due to variability of the hydrostatic pressure as a function of immersion depth. Further, immersion of the tip too deeply increases the surface area exposed to the sample liquid and increases the chance of droplets clinging to the exterior of the tip. Recommended immersion depths are given in Table 1.

Table 1 — Immersion depths of pipette tip during aspiration of sample liquid

Volume to be pipetted µl	Immersion depth below surface of the sample solution mm
0,1 to 1	1 to 2
1,1 to 100	2 to 3
101 to 1 000	2 to 4
>1 001	3 to 6

4.3.3.7 Speed of aspiration and dispense

The sample liquid should be aspirated smoothly, with a consistent, slow speed into the pipette tip. The optimal aspiration speed depends on the properties of the sample liquid, volume of the aspirated aliquot, size of the pipette and size of the pipette tip. The sample should be dispensed at a consistent speed from the pipette into the destination receptacle.

4.3.3.8 Tip position during aspiration and dispense

4.3.3.8.1 Aspiration

During the process of aspirating sample liquid into the pipette tip, the pipette should be held in a way that positions the pipette tip straight upright, and the pipette tip should not be allowed to touch the side walls or bottom of the sample vessel. Upon removal from the sample liquid vessel, the pipette tip should be inspected to ensure that no sample liquid droplets are clinging to the outside of the tip.

4.3.3.8.2 Dispense

When dispensing the sample into the destination receptacle, the pipette tip should be touched against the receptacle's side wall at an angle of 30° to 45° and above the liquid surface so that complete sample delivery can be achieved. Once the sample has been dispensed, the tip should be dragged along the receptacle's wall for 5 mm to 8 mm to ensure no droplet adheres to the tip when removed from the receptacle.

If it is not possible to dispense against the side wall of the destination receptacle, and the sample is dispensed into the liquid contained in the destination receptacle, below its surface, liquid from the destination receptacle can cling to the outside of the tip, or can accidentally be aspirated back up into the tip. If this technique is practiced, it should only be during forward-mode pipetting, and the blow-out function should be used if the pipette is designed with it. The tip should be immersed as shallowly as possible below the

liquid surface to avoid unintended delivery of sample liquid from the outside of the tip to the destination receptacle.

4.3.3.9 Pause after aspiration

The pipette tip should remain immersed below the surface of the sample liquid for a period of time after the plunger has reached its initial position after the completion of the aspiration stroke. For results with the best precision, this pause should be the same after each aspiration. The duration of this pause depends on the liquid properties, such as its viscosity, as well as on the volume of the aspirated liquid. Table 2 gives recommended pause times for aqueous solutions.

Table 2 — Duration of pause after aspiration of aqueous liquid

Volume of aspirated liquid µl	Pause after aspiration s
0,1 to 1	1
1,1 to 100	1
101 to 1 000	1
> 1 001	3 or longer [a]

[a] For pipettes with nominal volumes over 5 ml, the pause can last up to 5 s to allow for full equilibration of pressure.

4.3.3.10 Tip wiping

Wiping tips during pipetting is not recommended. Wiping the tip introduces the risk of wicking sample liquid out of the tip. Cross-contamination may jeopardize results and compromise the integrity of the sample and reagent. Touching the tip orifice can introduce large volumetric errors.

4.3.4 Using air-displacement pipettes for liquids with properties differing from water

4.3.4.1 General

Liquid properties for solutions exhibiting non-aqueous behaviour can vary considerably, depending on the nature of the liquid. Guidance in 4.3.4.2 to 4.3.4.6 is general and should be adjusted, depending on the extent of non-aqueous properties.

4.3.4.2 Viscous liquids

Positive displacement pipettes should be used for pipetting viscous liquids. When using air-displacement pipettes, reverse pipetting mode should be employed. Aspiration and dispense speeds should be slowed to allow consistent and continuous liquid flow in the pipette tip. Waiting times after aspiration and dispense should be increased to allow each cycle to be completed. The optimal aspiration and dispense speeds, and waiting times, depend on the viscosity of the liquid; the higher the viscosity, the slower speeds should be employed.

Wide-orifice and low-retention pipette tips may be used, if available, for the make and model of the pipette used. The pipette tip shall not be physically altered from its supplied design (e.g., by cutting the tip to achieve a larger orifice), as this alters its volumetric performance.

4.3.4.3 Biological solutions

Ensure homogeneity of the biological solution, particularly if not all constituents are fully soluble. Biological solutions can exhibit a variety of liquid properties, including increased viscosity, decreased surface tension, and/or constituents that can separate from the solution, either on the inside or outside of the tip, or as a layer on top of the aspirated liquid.

Positive displacement pipettes should be used for pipetting biological solutions with increased viscosity or components that can adhere to the inside of the tip. The recommendations for pipetting viscous liquids

should be followed (see 4.3.4.2). When using air-displacement pipettes, reverse pipetting mode should be employed.

When using air-displacement pipettes, wide orifice tips may be used, particularly when pipetting suspended cells.

4.3.4.4 Volatile liquids

Volatile liquids are characterized by an increased rate of evaporation, as compared to water, at normal temperatures due to their high vapour pressures. The evaporation rate of a liquid increases as the pressure above that liquid decreases. As a liquid is aspirated by a POVA, the evaporation rate increases, which increases the evaporation of liquid molecules into the dead air space inside the pipette tip. This increases the volume of gas in the dead air space, which in turn reduces the amount of liquid that can be aspirated inside the tip and can lead to loss of liquid from the tip (dripping). As a result, the transferred volume of a liquid with high vapour pressure can be lower than that of a liquid with lower vapour pressure. See 4.3.3.2 regarding the importance of pre-wetting tips.

The use of positive displacement pipettes or syringes is recommended for liquids with a high vapour pressure.

4.3.4.5 Liquids with surfactants

Surfactants (detergents) alter the surface tension of the liquid, which is one of the key liquid properties when using air-displacement pipettes. It is therefore recommended to use positive displacement pipettes. Liquids containing surfactants tend to develop foam when pipetted. When using air-displacement pipettes, appropriate measures (e.g., filter tips) should be used to prevent foam from entering the pipette shaft, and reverse pipetting mode should be employed.

4.3.4.6 Corrosive liquids

Strong mineral acids and bases, concentrated salt solutions, as well as organic solvents can interact with the materials of the pipette tip, pipette shaft, piston, or seals and gaskets. Liquids of corrosive nature should be pipetted carefully with filter tips, or with positive displacement pipettes of type D2. Care should be taken that no liquid or aerosol enters the pipette shaft.

Corrosive liquids and their vapours can lead to increased wear of pipette components, and the pipette should be inspected, maintained, and calibrated more frequently than when used with non-corrosive liquids.

4.3.5 Positive displacement pipettes

4.3.5.1 Pre-wetting of pipette tips

After fitting a new tip to the pipette, the desired volume of sample solution should be aspirated and dispensed at least twice. This process reduces a potential air gap between the aspirated liquid and the piston end.

The positive displacement tip should be inspected after it has been removed from the sample liquid container to verify that liquid did not pass over the piston seal.

4.3.5.2 Immersion depth

The orifice of the pipette tip should be immersed to the appropriate depth below the surface of the sample liquid and remain at this depth while aspirating sample liquid into the pipette tip. The orifice should be immersed at least 2 mm below the liquid surface, irrespective of the nominal volume of the positive displacement pipette.

4.3.5.3 Tip wiping

If necessary, for example, when pipetting highly viscous liquids, wipe the outside of the tip or the capillary with a clean medical wipe after aspiration of the liquid. It is crucial to not touch the orifice as sample liquid

can be wicked out of the tip, resulting in large volumetric errors. Cross-contamination may jeopardize results and compromise the integrity of the sample and reagent. It is recommended to choose a tissue which is lint-free and inert to the liquid being pipetted. The tissue should be disposed of in a safe, hygienic manner.

4.3.5.4 Dispense

Once the sample has been dispensed, the tip should be dragged along the receptacle's wall for 5 mm to 8 mm to ensure no droplet adheres to the tip when it is removed from the receptacle. Manufacturer's recommendations should be followed for achieving a regular pipetting cycle.

4.3.6 Burettes

4.3.6.1 Thermal equilibrium

The burette and the reservoir, already filled with liquid, should be acclimatised long enough to reach thermal equilibrium.

4.3.6.2 Liquids

Prior to using a burette with a particular liquid (e.g., viscous, volatile, corrosive, suspensions containing solid particles, etc.) the manufacturer's instruction manual and/or liquid data sheet should be consulted and checked for compatibility with the intended liquid.

The use of titrants that can form deposits shall be confirmed by the manufacturer's instruction manual because they can damage the burette components.

Strong mineral acids and bases, concentrated salt solutions, as well as organic solvents can interact with the materials of the burette and result in increased wear or damage to some components of the burette.

Aggressive liquids and their vapours can lead to increased wear of burette components and the burette should be inspected, maintained, and calibrated more frequently than when used with non-aggressive liquids.

When using the burette with liquids that can crystallize, the burette should remain filled with the liquid.

Completely cleaning the burettes directly after use can prevent possible damage from the used liquids.

4.3.6.3 Priming

The burette should be primed with the liquid before use in order to remove any air bubbles from the system. Priming also serves to facilitate the equilibration of temperature differences between the liquid, the burette's inner walls, and the plunger.

4.3.6.4 Other handling aspects

The liquid delivery velocity should be established according to the manufacturer's instructions as it can affect the delivered liquid quantity.

The first drops of liquid shall be discarded before starting the delivery if instructed to do so by the manufacturer. Liquid delivery should be achieved with the minimum number of interruptions to the liquid flow.

4.3.6.5 Maintenance

The condition of the valves and aspiration tubes shall be checked frequently. Please refer to 4.3.6.2 for potential types of damage. The manufacturer's instructions should be followed and applied by trained personnel.

4.3.7 Dilutors

The manufacturer's instructions for general maintenance of the dilutor should be followed, with particular attention to cleaning the liquid path.

The dilutor and the liquids to be used with the dilutor shall be in thermal equilibrium. Prior to use, the system should be primed to remove air pockets from all liquid lines and valves.

The dilutor should be cleaned frequently according to the manufacturer's instructions. Generally, cleaning with caustic or acidic solutions should be avoided.

The dilutor should not be allowed to run dry while using buffers or other salt solutions, as these substances can crystallize in the liquid path.

4.3.8 Dispensers

4.3.8.1 Thermal equilibrium

The dispenser, already filled with liquid, should be acclimatised long enough to reach thermal equilibrium.

4.3.8.2 Liquids

Prior to using a dispenser with a particular liquid (e.g., viscous, volatile, corrosive, suspensions containing solid particles) the manufacturer's instruction manual and/or liquid data sheet should be consulted and checked for compatibility.

The use of liquids that can form deposits shall be confirmed by the manufacturer's instruction manual because they can damage the dispenser components.

Strong mineral acids and bases, concentrated salt solutions, as well as organic solvents can interact with the materials of the dispenser and result in increased wear or damage to some components of the dispenser.

Corrosive liquids and their vapours can lead to increased wear of dispenser components and the dispenser should be inspected, maintained, and calibrated more frequently than when used with non-corrosive liquids.

When using the dispenser with liquids that can crystallize, the dispenser should remain filled with the liquid.

Cleaning the dispenser completely with distilled water directly after use can prevent possible damage from the used liquids.

4.3.8.3 Priming

The dispenser should be primed with the liquid before use in order to remove any air bubbles from the system. Priming also serves to facilitate the equilibration of temperature differences between the liquid, the dispenser's inner walls, and the plunger.

4.3.8.4 Other handling aspects

The liquid delivery velocity should be established according to the manufacturer's instructions as it can affect the dispensed liquid quantity.

4.3.8.5 Maintenance

The condition of the valves and aspiration tubes shall be checked frequently. Please refer to 4.3.8.2 for potential types of damage. The manufacturer's instructions should be followed and applied by trained personnel.

4.3.8.6 Single-stroke dispensers

Single-stroke dispensers can be mechanically or electronically operated.

4.3.8.7 Multi-dispensers (repeaters)

Multi-dispensers can be mechanically or electronically operated.

The first drops of liquid shall be discarded before starting the delivery if instructed to do so by the manufacturer. Liquid delivery should be achieved with the minimum number of interruptions to the liquid flow.

4.3.9 Syringes

4.3.9.1 General

Syringes may be used with a needle or a tube. For simplicity, this document only uses the term needle, but the same recommendations apply to syringes used with tubes.

4.3.9.2 Thermal equilibrium

The syringe and liquid should be in thermal equilibrium with the environment in order to obtain correct volume transfer results. Therefore, the syringe and the liquid should be acclimatised long enough to reach thermal equilibrium.

4.3.9.3 Priming

After fitting the syringe with the needle, it shall be primed with the liquid until no bubbles are present inside the syringe.

NOTE Some bubbles can be very small and not easy to detect.

4.3.9.4 Setting of the volume

Setting the syringe volume correctly is crucial for the accuracy of the delivered liquid quantity. The volume should be set by adjusting the plunger until the fiducial line corresponds to the graduation line of the volume to be delivered. If available, optical aids may be used to adjust the volume.

4.3.9.5 Immersion depth

The needle should be immersed to a suitable depth below the surface of the liquid and remain at this depth while aspirating liquid into the syringe. Immersing the syringe needle not deeply enough below the surface of the liquid can result in the aspiration of air.

4.3.9.6 Velocity of aspiration and delivery

The liquid should be aspirated with a smooth and consistently slow velocity into the syringe to avoid the introduction of air bubbles. The sample should be delivered with a slow and consistent velocity from the syringe into the receiving vessel.

4.3.9.7 Aspiration

During the process of aspiration of liquid, the syringe should be held straight upright, and the needle should not touch the side walls or the bottom of the vessel. Upon removal from the vessel and plunger adjustment to the desired volume, the syringe needle should be inspected to ensure that no liquid droplets are clinging to the outside of the needle.

4.3.9.8 Delivery

When delivering the liquid, the needle should touch the vessel wall above the liquid surface at an angle of 30° to 45°. Once the liquid has been delivered, the needle should be dragged along the vessel wall for 5 mm to 8 mm to ensure that no droplet adheres to the needle.

If it is not possible to dispense against the side wall of the destination receptacle, and the sample is dispensed into the liquid contained in the destination receptacle, below its surface, liquid from the destination receptacle can cling to the outside of the needle or can accidentally be aspirated back up into the needle. The needle should be immersed as shallowly as possible below the liquid surface to avoid unintended delivery of sample liquid from the outside of the needle to the destination receptacle.

4.3.9.9 Hand warming

Handling the syringe for extended periods of time can cause hand warming effects, which can lead to thermal disequilibrium, thus introducing volumetric errors. Heat transfer to the syringe can be mitigated by wearing gloves and only touching the flanges and top of the plunger.

4.3.9.10 Cleaning

Syringes should be cleaned immediately after use.

To avoid volume changes through glass erosion and destruction of graduations, syringes should be cleaned gently with detergents of low alkalinity. The manufacturer´s instructions should be followed.

5 User qualification and re-qualification

5.1 General

Volumetric performance is the ability to consistently deliver accurate volumes. For most piston-operated apparatus, in particular air-displacement pipettes, the volumetric performance is directly dependent on the technique employed by the user of the apparatus. Significant random errors, as well as systematic errors, in the volumetric performance of a POVA can be introduced by the technique employed by its user. With certain types of POVA, such as pipettes, the user and POVA form a system, and the overall volumetric performance is dependent on the POVA and its user.

5.2 User training

Persons who use, test, or calibrate POVA shall be trained on the correct use of the apparatus and should be provided with refresher training at regular intervals. Topics described in this document form a basis for user training and should be included. Training topics may be amended for individual circumstances and requirements.

NOTE Training for persons who use POVA for laboratory work can be different from training for persons who solely calibrate POVA.

Measurement procedures described in ISO 8655-6, ISO 8655-7, or ISO 8655-8 are suitable for evaluating the user when volumes are delivered by a calibrated POVA.

5.3 User competence

Users of POVA shall demonstrate the competence necessary to operate the relevant types of POVA at the relevant volumes for their work. The laboratory should establish and document the competence requirements for each POVA user, including qualification and training requirements.

User competence shall be assessed on a regular basis, at least every 12 months. The frequency and method of user competence assessment may be determined through a risk assessment of the user's work in the

laboratory. Tolerances required of the POVA user shall be determined by the laboratory's requirements. No less than four replicates per volume shall be tested.

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

5.4 User qualification

User qualification shall include training (5.2) and demonstrated competence (5.3) in the use of the specified POVA according to this document. A qualified user according to this document shall complete a qualification assessment on a regular basis, as determined by the risk assessment of the user's work, or at least every 12 months.

User qualification shall be carried out using a suitable method of testing, which shall be stated in any documentation according to 5.5.

5.5 Documentation of user qualification

The laboratory shall have procedures, and retain records, for all training, refresher training, competence assessment, and qualification of POVA users. A report of training or competence assessment may be issued to the user.

At least the following information shall be reported in the user training or user competence assessment report:

a) indication whether it is a training report or competence assessment report;

b) identification of the user;

c) in case of user training, the trainer shall be identified;

d) identification of the POVA used for training or skills assessment, including the type of tips or other exchangeable parts used with the piston-operated volumetric apparatus for the test;

e) test conditions under which the test was performed, including at least the ambient temperature and relative humidity of the room;

f) volumes at which the user was trained and/or assessed;

g) 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;

h) reference to ISO 8655-10 and the employed test method (e.g., by reference to ISO 8655-6, ISO 8655-8, or a specific test procedure in ISO 8655-7);

i) any deviation from this document;

j) volumetric measurement results for each delivered volume;

k) total number of replicate measurements made for each tested volume, and the number of measurement results used for statistical analysis;

l) date of test and duration of validity.

6 POVA qualification and re-qualification

6.1 General

Performance requirements shall be established for the POVA used to perform the laboratory's operational tasks. Regularly assessing these performance requirements shall ensure that the POVA is fit for its intended purpose.

Calibration, testing, and routine test requirements for the POVA shall be established to ensure continued adherence to the performance requirements.

It is best practice to determine the scope and frequencies of such requirements through a risk assessment.

6.2 Metrological performance requirements

6.2.1 POVA maximum permissible errors

ISO 8655-2, ISO 8655-3, ISO 8655-4, ISO 8655-5, and ISO 8655-9 include product specifications for POVA's maximum permissible systematic and random errors. These product specifications should be considered when selecting POVA so that the laboratory's performance requirements can be met.

6.2.2 Liquid handling process tolerances

Liquid handling process tolerances represent the maximum acceptable uncertainty in use of a single delivered volume. They take into account the error of the POVA, as well as the impact of the user, the liquid and the environmental conditions under which the POVA is used.

NOTE 1 Uncertainty in use of a single delivered volume is associated with a single delivered volume in daily use and includes both systematic and random errors of the POVA (see Clause 7).

NOTE 2 A POVA is fit for its intended use if its uncertainty in use of a single delivered volume is smaller than or equal to the liquid handling process tolerance.

NOTE 3 The liquid handling process tolerance is often expressed as a percentage of the nominal or selected volume.

EXAMPLE If a biological assay is performed with a required accuracy of 10 %, the laboratory can consider establishing a liquid handling process tolerance of, for example, 2 %. This way, the uncertainty contribution of the liquid delivery process is controlled and small compared to the overall requirement of the assay.

6.2.3 Establishing performance requirements

Achievable performance requirements should be set for POVA. They can be based on maximum permissible systematic and random errors, but may differ from the POVA product requirements given in ISO 8655-2, ISO 8655-3, ISO 8655-4, ISO 8655-5 and ISO 8655-9. Alternatively, liquid handling process tolerances can be used as performance tolerances. Regional, national, industry-specific, or organization-specific requirements for performance tolerances can apply.

NOTE 1 As an example, in the absence of known liquid handling process tolerances, CLSI QMS 23[5] recommends random error tolerances be limited to 1 % of the POVA's nominal volume at all selectable volumes, and systematic error tolerances be limited to 2 % of the nominal volume.

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

NOTE 2 Meeting manufacturer's performance specifications for POVA can be difficult, depending on user techniques, environmental conditions, age of the POVA, as well as its use and maintenance history.

6.3 Frequency

Metrological confirmation, including calibration or testing, shall be performed on a regular basis and at least once every 12 months. Routine tests shall be performed on a regular basis, preferably at shorter time intervals. The frequency shall be based on a risk assessment and determined so that the continued fitness of the POVA for its intended use is documented. Regional, national, industry-specific, or organization-specific requirements for the frequency of calibrations, testing and routine tests can apply.

In a risk assessment it is best practice to consider the laboratory's work or a specific application. The risk assessment includes an impact evaluation of a process outcome if incorrect liquid volumes are used, as well

as its probability of occurrence. The tighter a tolerance is, the larger is the probability of occurrence that an incorrect liquid volume is used.

6.4 Replicate measurements

6.4.1 General

Calibration and testing of POVA according to the reference measurement procedures described in ISO 8655-6 and ISO 8655-8, or according to ISO 8655-7, require at least ten replicate measurements for each volume setting. Routine tests may be performed with less than ten, but not less than four, replicate measurements per volume setting.

6.4.2 Impact of the number of replicate measurements on uncertainty

The uncertainty associated with the calculated random and systematic errors increases when the number of replicate measurements is reduced.

ISO/TR 16153 and ISO/TR 20461 provide information on how a decreasing number of replicate measurements increases the uncertainty. ISO/TR 16153:2023, Clause 11, and ISO/TR 20461:2023, Clause 11, describe the choice of an appropriate coverage factor based on the estimated effective degrees of freedom ν_{eff} using the Welch-Satterthwaite formula. The corresponding coverage factor k can then be determined from ISO/IEC Guide 98-3:2008, Table G.2; an excerpt of that table is shown in Table 3. Coverage factors shown in Table 3 for effective degrees of freedom between 1 and 10 are based on a coverage probability of 95,45 %.

Table 3 — Coverage factors k for different effective degrees of freedom ν_{eff}

ν_{eff}	1	2	3	4	5	6	7	8	9	10
k	13,97	4,53	3,31	2,87	2,65	2,52	2,43	2,37	2,32	2,28

For uncertainties of type A, such as the experimental standard deviation measured by n replicate measurements, the effective degrees of freedom are given by Formula (1):

$$\nu_{eff} = n - 1 \tag{1}$$

where:

ν_{eff} is the number of effective degrees of freedom;

n is the number of replicate measurements.

The use of less than 4 replicate measurements at each volume is not recommended as the confidence levels are severely reduced.

NOTE 1 For n = 10 or more replicates, k = 2 can be used by convention, assuming a normal distribution.

NOTE 2 Detailed examples for n = 10 replicates are described in ISO/TR 16153 and ISO/TR 20461.

EXAMPLE In this example, a routine test is carried out with n = 4 replicate measurements, with the goal to estimate the uncertainty in use of a single delivered volume according to Formula (A.3) of ISO/TR 20641:2023 or Formula (A.5) of ISO/TR 16153:2023. In this case, according to Table 3, the effective degrees of freedom are 3 and the coverage factor is k = 3,31.

6.5 Pass/fail decision rules

6.5.1 General

The decision rule for the determination of the pass/fail status of a POVA shall be agreed and documented.

6.5.2 No comparison to tolerances

The systematic error and random error for the volume measurements according to ISO 8655-6, ISO 8655-7, or ISO 8655-8 may be reported without comparison to performance tolerances and without determination of the pass/fail status of the POVA under test.

6.5.3 Pass/fail determination of calibration

The pass/fail status of a calibration shall be determined by assessing the systematic and random errors versus established tolerance limits. The expanded uncertainty of the mean delivered volume shall be estimated and include contributions from the measuring system, the POVA under test, and the liquid delivery process. Additionally, the measurement uncertainty in use of a single delivered volume can be estimated.

NOTE The approaches given in ISO/TR 16153 and ISO/TR 20461 meet the requirements for uncertainty estimations in 6.5.3. Guidance in the ISO/IEC Guide 98-3 is also applicable.

6.5.4 Pass/fail status of routine tests

For routine tests, the evaluation may be performed by assessing the systematic and random errors versus established tolerance limits. Alternatively, both errors may be combined into the uncertainty in use of a single delivered volume that is assessed versus the liquid handling process tolerance.

NOTE An example for a routine test evaluation is described in ISO/TR 20461:2023, A.3.3 and ISO/TR 16153:2023, A.3.3.

6.5.5 Pass/fail assessment

6.5.5.1 Simple acceptance rule

A binary simple acceptance rule may be applied for the determination of the pass/fail status. The impact of the number of replicates on the confidence level shall be considered.

6.5.5.2 Other acceptance rules

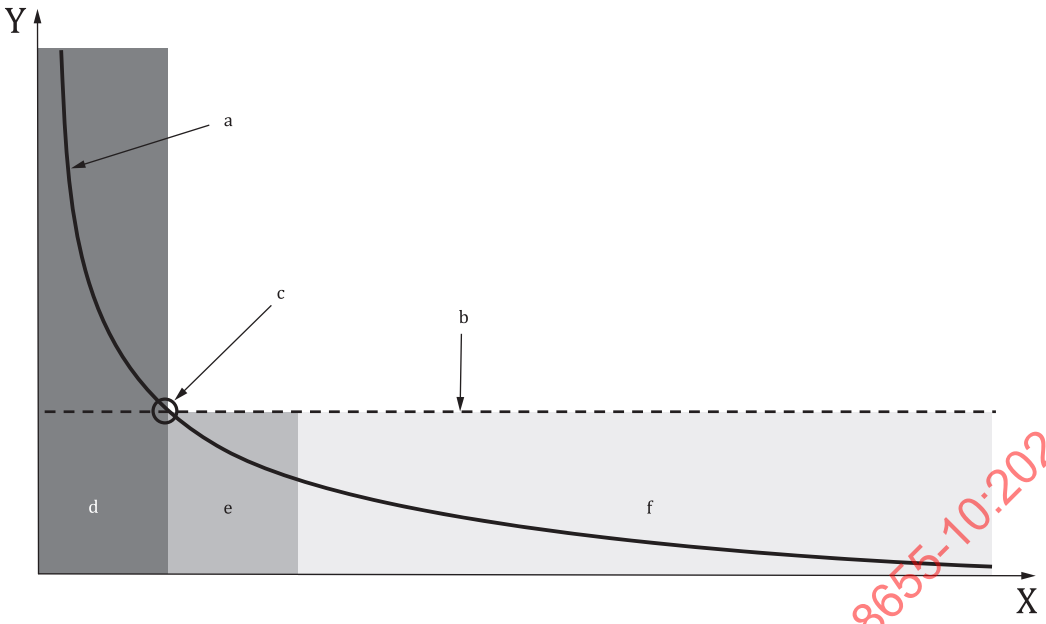
Guard bands and non-binary acceptance rules may be applied. These acceptance rules are described elsewhere, e.g., in ILAC Guide 8[6].

6.6 Safe liquid handling range

The volumetric performance of adjustable volume POVA is usually best at or near the nominal volume and worst at its lowest selectable volume.

The relative uncertainty in use of a single delivered volume increases with decreasing volumes. The point at which the relative uncertainty equals the liquid handling process tolerance represents the POVA's lower useable limit (see Figure 1). This is the minimum volume the POVA can still deliver with a given accuracy. When a POVA is used below this limit (Figure 1, range "d"), the uncertainties of the delivered volumes become larger than the acceptable process tolerance, resulting in potentially inaccurate results. When a POVA is used in the safe liquid handling range (Figure 1, range "f"), however, the uncertainties of the volumes are smaller than the required tolerance, yielding consistently accurate results. The POVA's limit, and thus its safe liquid handling range, depends on the chosen liquid handling process tolerance.

To account for uncertainty variations contributed by the POVA operator, consumables, liquid properties, environmental conditions or other uncontrollable factors in daily use, the known uncertainty may be multiplied by a safety factor. This safety factor provides an additional safety margin (Figure 1, range "e") between the POVA's lower useable limit and the safe liquid handling range.



Key

X selected volume, in µl
Y uncertainty of the delivered volume, in %
a relative uncertainty, in %
b liquid handling process tolerance, in %
c POVA limit, minimum useable volume, in µl
d volume range with uncertainty exceeding the process tolerance
e safety margin
f safe liquid handling range

Figure 1 — Safe liquid handling range

6.7 Maintenance

POVA are mechanical devices and require routine maintenance to ensure consistent performance and to prevent failures. The level and frequency of maintenance depend on the POVA, its use, and its user. The user manual should detail the manufacturer's recommended maintenance required and its frequency. It is best practice to determine maintenance frequency through a risk assessment.

At a minimum, the following shall be performed at least every 12 months:

— physical inspection for damage and functionality;

— leak test where appropriate;

— replacement and/or lubrication of parts susceptible to wear (e.g., seals, tip cones, etc.) according to recommendations given in the user manual;

— confirm correct mounting and ejection of disposable or reusable parts (e.g., pipette tips, syringe needles, etc.).

In case an as-found calibration is required, any maintenance should only be carried out after the as-found calibration has been performed. If maintenance includes an adjustment, it shall be followed by an as-left calibration to confirm performance.

NOTE An as-found calibration establishes the performance of the POVA prior to maintenance and/or adjustment. An as-left calibration establishes the performance of the POVA after maintenance and/or adjustment.

7 Uncertainty in use of a single delivered volume

Uncertainty of measurement is considered in detail in ISO/TR 16153 and ISO/TR 20461, based on the highly defined procedures in ISO 8655-6, ISO 8655-7, and ISO 8655-8. Use of a POVA in a routine laboratory (e.g., clinical or research laboratory) rather than a testing or calibration laboratory, and with differing environmental conditions and liquids, will inevitably provide results that differ from those obtained under reference conditions.

The concept of uncertainty in use of a single delivered volume is a method for the determination of accuracy in routine use. This means that one can assess added potential error and suitability for purpose by performing testing done by the user in their laboratory, i.e., in the environment in which the POVA is used. Testing POVA with the consumables used in regular use and under the conditions of regular use gives the closest correlation of metrological performance between calibration/testing and regular use.

The uncertainty in use of a single delivered volume is useful when assessing the accuracy of a delivered volume for a particular application and when evaluating POVA by routine testing. For routine testing, performed by the user and in the user's environment, the uncertainty in use of a single delivered volume can be assessed against the liquid handling process tolerance.

The uncertainty in use of a single delivered volume is larger than the uncertainty of the mean delivered volume because it includes both systematic and random errors of the deliveries (see ISO/TR 16153:2023, Annex A and ISO/TR 20461:2023, Annex A).

Due consideration should be given to the fact that calibrations are carried out using defined test liquids whilst the POVA in normal use is commonly not used with these test liquids. The differences that may be experienced in normal use, as compared to calibration results, should be determined.

Furthermore, the accuracy of delivered volumes may vary with the skillset and experience of the POVA users. To account for such differences in daily use, the uncertainty in use of a single delivered volume may be multiplied by a safety factor when being assessed against the liquid handling process tolerance.

Annex A
(informative)

Selection of pipettes and exchangeable parts

A.1 Pipettes

The following types of pipettes are available and commonly used in laboratories. For the sake of a complete overview of pipettes, the list in this subclause and Table A.1 also contain pipettes that are not piston-operated.

a) Piston-operated, air-displacement, fixed volume pipettes

b) Piston-operated, air-displacement, variable volume pipettes

c) Piston-operated, air-displacement, variable volume, multi-channel pipettes

d) Piston-operated, positive displacement, fixed volume pipettes (types D1 and D2, see A.2.3)

e) Piston-operated, positive displacement, variable volume pipettes (types D1 and D2, see A.2.3)

f) Piston-operated, manual and electronic multi-dispensers

NOTE Sometimes, these dispensers are colloquially referred to as “repeater or ratchet pipettes.”

g) Piston-operated, adjustable spacing multi-channel pipettes

h) Piston-operated, electronic single channel and multi-channel pipettes

i) Serological pipettes, made from glass or plastic, with a graduated volume scale – usually for volumes ≥1 ml

j) Volumetric glass pipettes with single calibration mark

k) Transfer pipettes made from glass (Pasteur pipettes) or plastic (transfer pipettes), with or without calibration marks

Table A.1 lists advantages and disadvantages of these types of pipettes.

Variable volume pipettes exhibit their most accurate volumetric behaviour at or near the pipette’s nominal volume. While adjustable volume pipettes sometimes allow a very wide range of volumes to be selected, using the pipette within its safe liquid handling range of volumes ensures delivery of sufficiently accurate volumes.

NOTE For example, it is preferable to pipette 20 µl of sample with a 2 µl to 20 µl adjustable volume pipette at its nominal volume, instead of using a 10 µl to 100 µl pipette set at 20 µl.

Table A.1 — Selection of pipette type

Pipette Type	Recommended Use	Advantages	Disadvantages
Air-displacement, fixed volume, single channel pipettes	All aqueous samples. Samples with low and medium viscosity. Samples with low vapour pressure.	Typically higher accuracy than variable volume pipettes. Prevents user error due to improper volume adjustment or accidental volume change if setting is not properly locked.	Fixed volume setting. Limited numbers of fixed volumes available from manufacturers.
Air-displacement, variable volume, single channel pipettes	All aqueous samples. Samples with low and medium viscosity. Samples with low vapour pressure.	One pipette can be used for a variety of volume settings.	Possibility of improper volume setting by user can reduce trueness. Possibility of accidental volume change when volume setting is not locked.
Air-displacement, variable volume, multi-channel pipettes	All aqueous samples. Samples with low and medium viscosity. Samples with low vapour pressure.	Suitable for work in multi-well plates.	Each channel is regarded as single channel pipette and is subject to calibration requirements. Systematic and random error specifications can be larger than for single channel models.
Positive displacement, fixed volume pipettes	All aqueous samples. Samples with medium and high viscosity. Samples with medium to high vapour pressure. Hot and cold samples.	Aspirated sample is completely dispensed from tip. Suitable for non-aqueous, viscous, biological, and other samples which are difficult to pipette with air-displacement pipettes.	Single volume per pipette. Pipette tips are more expensive than those for air-displacement pipettes. Risk of cross contamination of samples unless the tip is disposed of between samples.
Positive displacement, variable volume pipettes	All aqueous samples. Samples with medium and high viscosity. Samples with medium to high vapour pressure. Hot and cold samples.	Aspirated sample is completely dispensed from tip. Suitable for non-aqueous, viscous, biological, and other samples which are difficult to pipette with air-displacement pipettes. Useful for all volumes within the safe pipetting range.	Pipette tips are more expensive than those for air-displacement pipettes. Risk of cross contamination of samples unless the tip is disposed of between samples.
Electronic air-displacement pipettes	All aqueous samples. Samples with low and medium viscosity. Samples with low vapour pressure.	Typically better repeatability (increased precision) than manually operated pipettes. Some pipettes can be programmed to deliver multiple dispenses from one aspirated aliquot of sample.	Electronic pipettes are typically more expensive than manual pipettes.
Multi-dispensers	All aqueous samples. Samples with low, medium, or high viscosity. Samples with low or high vapour pressure.	Suitable for repetitive delivery of the same volume without the need for aspiration after each delivery.	Trueness and precision can be inferior compared to single delivery pipettes.