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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

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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Contents

Page

Foreword

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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 practice

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 in 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.