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Gravimetric Calibration and Testing of Piston-Operated Volumetric Apparatus (POVA) According to Part 6 and 7 of the ISO 8655:2022

How to Perform a Norm Compliant Calibration or Test

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Abstract

Reliable and reproduceable liquid handling results depend crucially on the regular test and calibration of the instruments. The globally most accepted International Standardization Organization (ISO) standard that details requirements for producing and in-use control of piston-operated volumetric apparatus (POVA) is the ISO 8655.

The aim of this white paper is to present the requirements for gravimetric calibration and testing of piston-operated volumetric apparatus (POVA) according to part 6 and part 7 of the ISO 8655 revised in 2022.

This white paper presents the differences between calibration and testing according to chapters 6 and 7, describes the testing method as well as testing environment, testing equipment and reporting requirements.

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Introduction

Liquid handling products are used daily in most laboratories to prepare samples, controls, and assay reagents. As mechanical and electronical liquid handling products affect virtually every experiment, accuracy and repeatability are extremely important. Metrological confirmation by calibration or testing of all piston-operated volumetric apparatus (POVA) shall therefore be performed on a regular basis to ensure that the apparatus meets the requirements for its intended use.

The most important international standard on piston-operated volumetric apparatus (POVA) is the ISO 8655. It is applicable to pipettes, burettes, dilutors, dispensers and manually operated precision laboratory syringes for volumes up to two liters.

The ISO 8655 standard was developed and published by the International Organization for Standardization (ISO), an international, independent, non-governmental organization. In 2022 a second edition of the ISO 8655 was published. The ISO 8655:2022 series has now nine parts (a part 10 is in preparation) and replaces the first edition from 2002.

ISO 8655 series is addressing the needs of POVA manufacturers as well as calibration laboratories and POVA users performing calibration or tests on POVAs.

The aim of this white paper is to describe the requirements for a gravimetric calibration or test according to part 6 and part 7 of the ISO 8655:2022. This includes testing methods, testing environment, testing equipment, and reporting requirements.

Part 6 and Part 7 of the ISO 8655:2022

Part 6 and 7 of the ISO 8655:2022 specifies gravimetric measurement procedures for the determination of volume of piston-operated volumetric apparatus (POVA).



Figure 1: Gravimetric Calibration of a Pipette on a Single Channel Balance

Part 6 of the ISO 8655 describes the gravimetric reference measurement procedure for the determination of volume.

Conformity with Part 6 can only be given if all the requirements described in this part are met. Part 7 specifies alternative measurement procedures for the determination of volume. Annex A of part 7 of the ISO is entitled with "Gravimetric procedure".

Both in part 6 and part 7 the test procedures apply to the entire system which comprises the basic apparatus and all disposable (e.g.: pipette tips) or reusable parts that are selected for use with the apparatus. This means calibration and test results according to the ISO 8655 are only valid for the entire system. Thus, when different types of disposable or reusable parts or disposable or reusable parts from different manufacturers are used, different systems are in use, and each requires its own calibration or testing.

Part 6 and Part 7 of the ISO 8655 distinguish between calibration, test, and routine testing. All are defined as a set of operations that establish the relationship between the delivered volume and the corresponding selected volume of the apparatus. The difference between calibration and test according to the ISO 8655 is that the calculation of the measurement uncertainties is required for a calibration, while this is optional when testing a POVA. As measurement results are, strictly speaking, incomplete if they are not accompanied by a statement of the associated measurement uncertainty and measurement uncertainty is also crucial in measurement comparison and measurement traceability, calibration has a much higher value than just testing.

According to the ISO 8655:2022 a metrological confirmation of a POVA shall be performed on a regular basis to ensure that the apparatus meets the requirements for its intended use. Such a metrological confirmation can be given by both calibration and test and as well under Part 6 and Part 7 of the ISO 8655.

The ISO 8655:2022 clearly states that an as-found calibration or test should be carried out and a metrological confirmation should be considered before and after the maintenance or repair of a POVA.

While the main target of calibration and test lays at the metrological confirmation routine testing shall be performed at shorter time intervals than metrological confirmations and follows Part 7 of the ISO 8655.

	ISO 8655-6	ISO 8655-7	ISO 8655-7 A.2
Test Types	Calibration, Test	Calibration, Test, Routine testing	Calibration, Test, Routine testing
Metrological confirmation	Yes	Yes, if the measurement procedure is validated by comparison to the reference procedure	Yes, if the measurement procedure is validated by comparison to the reference procedure and at least 10 measurements per volume are taken

Balance Used for Gravimetric Calibration and Test

Gravimetric calibration and tests are performed by dispensing the test liquid several times on a balance. Single-channel balances can be used for calibration and test of single and multi-channel instruments while a multi-channel balance shall only be used for multi-channel pipettes.

The minimum requirements for the balance used for calibration and test are the same under Part 6 and Part 7 of the ISO. The following minimum requirements need to be met:

Nominal Volume of the POVA	Resolution (<i>d</i>) (mg)	Repeatability (<i>s</i>) (mg)	Expanded Uncertainty in use (U_{gi} [W]) (mg)
$0.5 \mu\text{L} \leq V < 20 \mu\text{L}$	0.001 ^a 0.01 ^b	0.006 ^a 0.03 ^b	0.012 ^a 0.06 ^b
$20 \mu\text{L} \leq V < 200 \mu\text{L}$	0.01	0.025	0.05
$200 \mu\text{L} \leq V \leq 10 \text{ mL}$	0.1	0.2	0.4
$10 \text{ mL} < V \leq 1,000 \text{ mL}$	1	2	4
$1,000 \text{ mL} < V \leq 2,000 \text{ mL}$	10	10	40

^a Single-channel balance

^b Multi-channel balance (to be used below 20 μL only if the expanded uncertainty in use is less than ¼ of the maximum permissible systematic error of the apparatus)

Resolution is quite simple – when knowing the nominal volume of the POVA the needed resolution | scale interval (*d*) of the balance can directly be taken from the table above.

An evaluation if the balance meets the requirements on repeatability (*s*) and the expanded uncertainty in use (U_{gi} [W]) is only possible by taking these values from an actual calibration certificate of the balance. It is important that the balance has been calibrated at the place of use and that both values, the repeatability (*s*) as well as the expanded uncertainty in use are stated in the calibration certificate.

In particular the expanded measurement uncertainty in use (U_{gi} [W]) is not specified on calibration certificates from all service providers. It is represented by a formula and allows the measurement uncertainty to be calculated for all loads. In contrast to the measurement uncertainty that is given on a calibration certificate for the calibration result, the formula

of the expanded uncertainty in use takes additional uncertainty contributions into account, such as effects when taring the balance, environmental influences or rounding effects.

Calibration certificates from Sartorius that follow the EURAMET cg-18 guideline do always state the expanded uncertainty in use. With a special attachment to the calibration certificate the Sartorius service can provide a document confirming the use of the balance for calibrations of POVAs of different nominal volumes.

Test Equipment Used for Gravimetric Calibration and Test

The minimum requirements for the test equipment to measure the environmental condition during calibration or test slightly differ between Part 6 and Part 7 of the ISO 8655:2022. The following minimum requirements shall be met:

	ISO 8655-6	ISO 8655-7	ISO 8655-7 A.2
Test equipment	All measurements done by test equipment shall be traceable to the International System of Units (SI) and shall meet the uncertainty requirements of ISO 8655-6	Shall be chosen such that the required uncertainty of measurement can be obtained	
Air temperature	Minimum requirements to be met: - Resolution: 0.1 °C - Expanded uncertainty of measurement ($k = 2$): 0.3 °C		
Water temperature	Minimum requirements to be met: - Resolution: 0.1 °C - Expanded uncertainty of measurement ($k = 2$): 0.2 °C	Minimum requirements: - Resolution: 0.1 °C - Expanded uncertainty of measurement ($k = 2$): 0.3 °C	
Air humidity	Minimum requirements to be met: - Resolution: 1% relative humidity - Expanded uncertainty of measurement ($k = 2$): 5% relative humidity		
Air pressure	Minimum requirements to be met: - Resolution: 0.1 kPa - Expanded uncertainty of measurement ($k = 2$): 1 kPa		
Test liquid	Minimum requirements to be met: Distilled or deionized water conforming to at least grade 3 as specified in ISO 3696:1987		

Test Conditions

The environmental conditions need to be measured and reported during gravimetric calibration and test of a POVA. The following environmental conditions must be met:

	ISO 8655-6	ISO 8655-7	ISO 8655-7 A.2
Test conditions in test room	- Draft free - Stable environment - Relative Humidity: 45% – 80 % relative humidity - Temperature: (20 ± 3) °C - Air pressure: to be recorded - Temperature variation during test ≤ 0.5 K	- Draft free - Stable environment - Relative Humidity: to be recorded - Temperature: to be recorded - Air pressure: to be recorded - Temperature variation during test ≤ 0.5 K	
Test liquid	The water temperature shall be within 0.5 K of ambient air temperature	Test liquid shall be acclimatized to the test room temperature	Not specified

Test Procedure

Depending on the part of the ISO 8655 and the test type the requirements on the measurement procedure differ. The following requirements are given under the different parts of the ISO and for the different test types:

	ISO 8655-6	ISO 8655-7	ISO 8655-7 A.2
Test volumes	Calibration and test At least at the following 3 volumes: ▪ nominal volume ▪ 50 % of the nominal volume ▪ The lower limit of the usable volume range or 10% of the nominal volume (whichever is greater).	Calibration and test At least at the following 3 volumes: ▪ nominal volume ▪ 50 % of the nominal volume ▪ The lower limit of the usable volume range or 10% of the nominal volume (whichever is greater). Routine testing Fewer than 3 volumes might be tested	All test types At least 1 volume
Number of measurements	Calibration and test At least 10 measurements per volume	Calibration and test At least 10 measurements per volume Routine testing At least 4 measurements per volume	All test types At least 4 measurements per volume
Tip change	At least once per volume	At least once per volume	No tip change required
Specific requirement when testing a multi-channel pipettes	▪ All channels must be tested individually ▪ All channels shall be tested on a multi-channel balance in parallel ▪ When testing on a single-channel balance test liquid shall be aspirated by all channels together. The volume of the channel to be measured shall be delivered into the weighing vessel, while the volumes from all other channels shall be discarded		



Figure 2: Gravimetric Calibration on a Speedcal Mobile Multi Channel Balance

Test Evaluation and Reporting of Results

	ISO 8655-6	ISO 8655-7	ISO 8655-7 A.2
Volume calculation	The corrected weighing values shall be converted to volume by using the general formula 2 according to ISO 8655-6 or using the Z correction table		
Systematic error of measurement	$e_s = \bar{V} - V_s$ e_s is the systematic error of measurement, expressed in units of volume V_s is the selected test volume at the POVA under test $\bar{V}$ is the average of the measured volume		
Random error of measurement	$s_r = \sqrt{\frac{\sum_{i=1}^n (V_i - \bar{V})^2}{n-1}}$ where s_r is the standard deviation, expressed in units of volume		
Tolerances	Results can be compared against following tolerances: - ISO 8655 (given in the technology related chapters of the ISO) - Manufacturer - Own customer requirements		
Reporting of results	- Identification of the POVA - Identification of tips exchangeable parts - Basis of the test (Ex) or (In) - Reference temperature and cubic thermal expansion coefficient γ, if a correction for cubic thermal expansion of the POVA is made - Test conditions under which the test was performed - Volumetric measurement results for each delivered volume - Total number of replicate measurements made per selected volume - Systematic and random measurement errors - Tolerances to which the test results are compared, if applicable - Expanded uncertainty of the mean delivered volume, for each selected volume and channel, if required - Date of the test - Identification of the operator performing the test - Reference to ISO 8655-6:2022 - Any variation from the reference measurement procedure specified under ISO 8655-6 - Reference to the formula used to convert weighing values into volume - Reference to ISO 8655-7:2022 - Any deviation from the employed procedure described under ISO 8655-7 - Type of test liquid - Recommendation for the next test date (if agreed upon) - Reference to ISO 8655-7:2022, A.2 - Any deviation from the employed procedure described under ISO 8655-6 - Type of test liquid - Recommendation for the next test date (if agreed upon)		

Conclusion and Sartorius Recommendations

- Testing and calibration are only valid for the entire system (basic apparatus and all disposable or reusable parts) – therefore when using different kinds of disposable or reusable parts with your POVA the test or calibration must be performed separately per entire system
- Balances that are used for calibration and test of POVA need to meet the requirements on resolution | scale interval (d), repeatability (s) and the expanded uncertainty in use (U_g [W])
- A metrological confirmation should be considered before and after the maintenance or repair of a POVA.
- When performing calibration or test the requirements for the balance and all other test equipment must be met
- When performing a calibration, the calculation and reporting of measurement uncertainties is required
- Test and calibration according to part 6 and 7 of the ISO 8655:2022 require at least one tip change per measured volume
- The test results can be compared against ISO 8655:2022, manufacturer, or customer tolerance settings

References

ISO 8655:2022 Piston-operated volumetric apparatus

Part 1: Terminology, general requirements and user recommendations

Part 2: Pipettes

Part 6: Gravimetric reference measurement procedure for the determination of volume

Part 7: Alternative measurement procedures for the determination of volume

International Organization for Standardization, Geneva, Switzerland

Version History		
Version	Date	Changes
01	February 2023	Initial Version
02	April 2023	Required Balance Load

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