INTERNATIONAL STANDARD

ISO 23783-2

First edition
2022-08

Automated liquid handling systems —

Part 2:
Measurement procedures for the determination of volumetric performance

Systèmes automatisés de manipulation de liquides —

Partie 2: Procédures de mesure pour la détermination des performances volumétriques

Reference number
ISO 23783-2:2022(E)

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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 documents 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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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

This first edition of ISO 23783-2, together with ISO 23783-1 and ISO 23783-3, cancels and replaces IWA 15:2015.

A list of all parts in the ISO 23783 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

Globalization of laboratory operations requires standardized practices for operating automated liquid handling systems (ALHS), communicating test protocols, as well as analysing and reporting of performance parameters. IWA 15:2015 was developed to provide standardized terminology, test protocols, and analytical methods for reporting test results. The concepts developed for, and described in, IWA 15 form the foundation of the ISO 23783 series.

Specifically, this document addresses the needs of:

— users of ALHS, as a basis for calibration, verification, validation, optimization, and routine testing of trueness and precision;

— manufacturers of ALHS, as a basis for quality control, communication of acceptance test specifications and conditions, and issuance of manufacturer's declarations (where appropriate);

— test houses and other bodies, as a basis for certification, calibration, and testing.

The tests established in this document should be carried out by trained personnel.

Automated liquid handling systems —

Part 2: Measurement procedures for the determination of volumetric performance

1 Scope

This document specifies procedures for the determination of volumetric performance of automated liquid handling systems (ALHS), including traceability and estimations of measurement uncertainty of measurement results.

This document is applicable to all ALHS with complete, installed liquid handling devices, including tips and other essential parts needed for delivering a specified volume, which perform liquid handling tasks without human intervention into labware.

NOTE For terminology and general requirements of automated liquid handling systems, see ISO 23783-1. Determination, specification, and reporting of volumetric performance of automated liquid handling systems is described in ISO 23783-3.

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 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 8655-6, *Piston-operated volumetric apparatus – Part 6: Gravimetric reference measurement procedure for the determination of volume*

ISO 23783-1, *Automated liquid handling systems — Part 1: Terminology and general requirements*

ISO 23783-3, *Automated liquid handling systems — Part 3: Determination, specification, and reporting of volumetric performance*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 23783-1 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 Abbreviated terms

For the purposes of this document, the abbreviated terms given in ISO 23783-1 apply.

5 Measurement methods

5.1 Overview of methods suitable for measuring ALHS performance

When choosing a test method for an ALHS, its suitability for the specific test situation shall be evaluated. This evaluation shall consider the systematic and random error requirements of the ALHS to which the test method is being applied. The selected test method shall be adequate to evaluate whether the ALHS performance is fit for its intended purpose.

NOTE 1 Fitness for purpose is a foundational concept and closely related to the process of metrological confirmation as described in ISO 9000 and ISO 9001.

The test method shall have a sufficiently small measuring system uncertainty (MSU) for the specific test situation. The MSU should be determined in accordance with a suitable approach (see 8.2 for more detail).

NOTE 2 The measurement model approach for estimating MSU is described in ISO/IEC Guide 98-3 and the measurement system approach is described in EURACHEM/CITAG Guide CG 4 [4].

Table 1 is intended to provide an overview of methods suitable for determining the volumetric performance of ALHS. It provides cross-references between the method abstracts from 5.2 to 5.5, and the corresponding procedures in Annexes B to I. It further describes the volume ranges, plate and liquid types which can be used for testing ALHS performance with a given method. It also lists typical systematic and random errors achievable if a test procedure is exactly followed as described in its respective annex. The suitability of a method for a given test situation may also be determined by the required equipment or environmental conditions under which it needs to be carried out.

Only key test equipment is listed in Table 1, while test equipment to monitor liquid and air temperatures, relative humidity, and barometric pressure is required for each procedure, as specified in the corresponding annexes.

Table 1 — Test methods for ALHS

Method Ref.	Method	Liquid type	Plate type wells	Volume range µl	Typical systematic error [a] %	Typical random error [a] %	Environmental conditions [b]	Test equipment
Photometric methods								
5.2.1 Annex B	Dual-dye ratiometric photometric method	Aqueous, DMSO [c]	96	0,1 to 350,0	2,0 to 3,0	0,15 to 0,25	Temperature: Aqueous: 15 °C to 30 °C DMSO [c]: 19 °C to 30 °C RH [d]: 20 % to 90 %	— Microplate absorbance reader capable of measuring absorbance at 520 nm and 730 nm; — dimensionally characterized 96- or 384-well microplates with optically clear bottom; — calibration plate for plate reader; — microplate shaker; — balance; — spectrophotometer capable of measuring absorbance at 520 nm and 730 nm; — pH meter; — volumetric flasks.
			384	0,01 to 55,0	2,5 to 5,5	0,35 to 0,55		

[a] Typically, larger test volumes lead to smaller errors.

[b] The minimum temperature of the test environment shall be above the melting point of the test liquid, ensuring that it will not solidify at any point during the test. The relative humidity of the test environment shall be non-condensing.

[c] Dimethylsulfoxide.

[d] Relative humidity.

Table 1 *(continued)*

Method Ref.	Method	Liquid type	Plate type wells	Volume range µl	Typical systematic error [a] %	Typical random error [a] %	Environmental conditions [b]	Test equipment
5.2.2 Annex C	Single-dye photometric method	Aqueous	96	1,0 to 100,0	3	1,5	Temperature: 15 °C to 30 °C RH [d]: 40 % to 70 %	— Microplate absorbance reader capable of measuring absorbance at 492 nm and 620 nm; — 96- or 384-well microplates with optically clear bottom; — balance; — magnetic stirrer; — microplate shaker; — pH meter; — manual pipettes; — volumetric flasks.
			384	0,25 to 20,0	3	1,5		

[a] Typically, larger test volumes lead to smaller errors.

[b] The minimum temperature of the test environment shall be above the melting point of the test liquid, ensuring that it will not solidify at any point during the test. The relative humidity of the test environment shall be non-condensing.

[c] Dimethylsulfoxide.

[d] Relative humidity.

Table 1 *(continued)*

Method Ref.	Method	Liquid type	Plate type wells	Volume range µl	Typical systematic error [a] %	Typical random error [a] %	Environmental conditions [b]	Test equipment
5.2.3 Annex H	Fluorescence method	Aqueous,	384	0,001 to 0,015	<8	<8	Temperature: 17 °C to 27 °C RH [d]: non-condensing	— Microplate fluorescence reader with excitation wavelength at 494 nm and emission analysis at 521 nm; — 384- or 1536-well fluorescence microplates; — balance; — bulk liquid dispenser or multi-channel pipette; — microplate shaker; — pH meter; — manual pipettes; — volumetric flasks.
		DMSO [c]	1 536	0,001 to 0,015	<8	<8		

[a] Typically, larger test volumes lead to smaller errors.

[b] The minimum temperature of the test environment shall be above the melting point of the test liquid, ensuring that it will not solidify at any point during the test. The relative humidity of the test environment shall be non-condensing.

[c] Dimethylsulfoxide.

[d] Relative humidity.

Table 1 *(continued)*

Method Ref.	Method	Liquid type	Plate type wells	Volume range µl	Typical systematic error [a] %	Typical random error [a] %	Environmental conditions [b]	Test equipment
Gravimetric methods								
5.3.1 Annex D	Single channel analysis	Any	n/a	0,5 to <20	≤1,4	≤0,6	Temperature: 17 °C to 30 °C RH [d]: 45 % to 80 %	— Balance; — density meter; — anti-electrostatic equipment; — anti-vibration table; — temperature- and humidity control for environment; — draft shield or draft-free environment for balance.
				20 to <200	≤0,9	≤0,3		
				200 to 1 000	≤0,9	≤0,3		
5.3.2 Annex E	Regression analysis	Any	n/a	<0,015	20 to 50	<10	Temperature: 17 °C to 27 °C RH [d]: 45 % to 80 % Barometric pressure: 600 hPa to 1 100 hPa	— Balance; — density meter with 6 decimal places; — anti-electrostatic equipment; — anti-vibration table; — temperature- and humidity control for environment; — draft shield or draft-free environment for balance.
				0,015 to <0,050	2 to 5	2,5 to 5		
				0,050 to 1	0,5 to 2	<0,5		

[a] Typically, larger test volumes lead to smaller errors.

[b] The minimum temperature of the test environment shall be above the melting point of the test liquid, ensuring that it will not solidify at any point during the test. The relative humidity of the test environment shall be non-condensing.

[c] Dimethylsulfoxide.

[d] Relative humidity.

Table 1 *(continued)*

Method Ref.	Method	Liquid type	Plate type wells	Volume range µl	Typical systematic error [a] %	Typical random error [a] %	Environmental conditions [b]	Test equipment
Photometric/gravimetric hybrid method								
5.4 Annex F	Tartrazine as chromophore	Aqueous	96	1,0 to 300,0	0,2 to 0,8	0,5 to 1,0	Temperature: 17 °C to 30 °C Temperature stability: < ±0,5 °C RH [d]: 45 % to 80 % RH [d] stability: < ±10 %	— Balance; — microplate absorbance reader capable to measure absorbance at the following wavelengths, depending on the chromophore used: — 4-nitrophenol: 405 nm and 620 nm, — Tartrazine: 450 nm and 620 nm, — Orange G: 492 nm and 620 nm — microplate shaker; — 96- or 384-well microplates with optically clear bottom; — manual pipettes; — centrifuge tubes 1,5 ml; — anti-vibration table; — temperature- and humidity control for environment; — draft shield or draft-free environment for balance.
			384	1,0 to 20,0	0,4 to 1,0	0,9 to 1,5		
	4-nitrophenol as chromophore	Aqueous	96	10 to 1 000 5 to 250	<1 to 5	1 to 2		
			96, 384	1 to 60 0,5 to 25	<1 to 5	1 to 2		
			384, 1 536	0,1 to 8	<2 to 10	2 to 5		
	Orange G as chromophore	Aqueous	96	1 to 100	<1 to 5	1,5		
			384	1 to 50	<1 to 5	1,5		

[a] Typically, larger test volumes lead to smaller errors.

[b] The minimum temperature of the test environment shall be above the melting point of the test liquid, ensuring that it will not solidify at any point during the test. The relative humidity of the test environment shall be non-condensing.

[c] Dimethylsulfoxide.

[d] Relative humidity.

Table 1 *(continued)*

Method Ref.	Method	Liquid type	Plate type wells	Volume range µl	Typical systematic error [a] %	Typical random error [a] %	Environmental conditions [b]	Test equipment
Dimensional methods								
5.5.1 Annex G	Optical image analysis of droplets	Any	n/a	Free flying droplets of V <0,5 µl	<5	<2	Temperature: (20 ± 3) °C or (27 ± 3) °C RH [d]: 50 % to 80 %	— Stroboscopic camera or high-speed camera; — automatic image detection software.
5.5.2 Annex I	Optical image analysis of capillaries	Any	n/a	0,1 to 1,0	<10	<7	Temperature: 15 °C to 35 °C RH [d]: 15 % to 90 %	— Flatbed scanner; — image analysis software; — specialized plates with capillaries.
				>1,0 to 1 000	<5	<4		

[a] Typically, larger test volumes lead to smaller errors.

[b] The minimum temperature of the test environment shall be above the melting point of the test liquid, ensuring that it will not solidify at any point during the test. The relative humidity of the test environment shall be non-condensing.

[c] Dimethylsulfoxide.

[d] Relative humidity.

5.2 Photometric methods

5.2.1 Dual-dye ratiometric photometric method

This method allows the determination of volumes of aqueous test liquids from 0,1 µl to 350 µl in 96-well plates, and from 0,01 µl to 55 µl in 384-well plates. Volumes of dimethylsulfoxide (DMSO)-based test liquids can be determined from 0,11 µl to 10 µl in 96-well plates, and from 0,01 µl to 2,5 µl in 384-well plates.

This method is suitable to determine the performance of ALHS with up to 384 channels. The operating environment for this method is 15 °C to 30 °C (19 °C to 30 °C for DMSO liquids), and it is not dependent on the ambient relative humidity and barometric pressure at the test location. Further information on the effect of relative humidity and barometric pressure on this method can be found in Reference [5].

Traceability of the measurement results to the International System of Units (SI) is achieved through the use of a calibrated microplate absorbance reader, dimensionally characterized microplates, calibrated balance, and calibrated volumetric glassware.

The procedure for the dual-dye ratiometric photometric method specified in Annex B shall be followed.

5.2.2 Single-dye photometric method

This method is suitable for evaluating the volumetric performance of ALHS with up to 384 channels using aqueous test liquids. Volumes from 1 µl to 100 µl can be measured in 96-well plates, and from 0,25 µl to 20 µl in 384-well plates.

Traceability of the measurement results to the SI is achieved through the use of a calibrated balance, calibrated pipettes, a calibrated microplate absorbance reader, and calibrated volumetric glassware.

The procedure for the single-dye photometric method specified in Annex C shall be followed.

5.2.3 Fluorescence method

This method is suitable to evaluate the volumetric performance of ALHS delivering volumes smaller than 15 nl. The fluorescence of the test liquid of fluorescein in DMSO is measured in 384-well or 1536-well microplates, which are specifically suited for fluorescence measurements.

This method is intended to be used for non-contact liquid delivery devices (e.g. acoustic, dispensing valves, or inkjet-type technology) that deliver the liquid volume as free flying droplets or jets into the wells of the microplate.

Traceability of the measurement results to the SI is achieved through the use of a calibrated fluorescence microplate reader, calibrated balance, calibrated pipettes, and calibrated volumetric glassware.

The procedure for the fluorescence method specified in Annex H shall be followed.

5.3 Gravimetric methods

5.3.1 Single channel method

This method describes the apparatus, procedure and reference material for recording measurements with the gravimetric method. A single pan balance is used to take a measurement from a single channel at a time. The following accommodations shall be made:

— placement of the balance and the weighing vessel which reduce draft and vibrations to a suitable level;

— control of the environmental conditions affecting the mass to volume conversion of the measurement (temperature and relative humidity);