



BSI Standards Publication

Automated liquid handling systems

Part 2: Measurement procedures for the determination of volumetric performance

National foreword

This British Standard is the UK implementation of EN ISO 23783-2:2023. It is identical to ISO 23783-2:2022. It supersedes BS ISO 23783-2:2022, which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee LBI/1/2, Laboratory Equipment.

A list of organizations represented on this committee can be obtained on request to its committee manager.

Contractual and legal considerations

This publication has been prepared in good faith, however no representation, warranty, assurance or undertaking (express or implied) is or will be made, and no responsibility or liability is or will be accepted by BSI in relation to the adequacy, accuracy, completeness or reasonableness of this publication. All and any such responsibility and liability is expressly disclaimed to the full extent permitted by the law.

This publication is provided as is, and is to be used at the recipient's own risk.

The recipient is advised to consider seeking professional guidance with respect to its use of this publication.

This publication is not intended to constitute a contract. Users are responsible for its correct application.

© The British Standards Institution 2023
Published by BSI Standards Limited 2023

ISBN 978 0 539 26431 9

ICS 17.060; 71.040.20

Compliance with a British Standard cannot confer immunity from legal obligations.

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 August 2022.

Amendments/corrigenda issued since publication

Date	Text affected
31 October 2023	This corrigendum renumbers BS ISO 23783-2:2022 as BS EN ISO 23783-2:2023

English Version

Automated liquid handling systems - Part 2: Measurement procedures for the determination of volumetric performance (ISO 23783-2:2022)

Systèmes automatisés de manipulation de liquides -
Partie 2: Procédures de mesure pour la détermination
des performances volumétriques (ISO 23783-2:2022)

Automatisierte Flüssigkeitsdosiersysteme - Teil 2:
Messverfahren zur Bestimmung der volumetrischen
Leistung (ISO 23783-2:2022)

This European Standard was approved by CEN on 25 September 2023.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

European foreword

The text of ISO 23783-2:2022 has been prepared by Technical Committee ISO/TC 48 "Laboratory equipment" of the International Organization for Standardization (ISO) and has been taken over as EN ISO 23783-2:2023 by Technical Committee CEN/TC 332 "Laboratory equipment" the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by April 2024, and conflicting national standards shall be withdrawn at the latest by April 2024.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

Any feedback and questions on this document should be directed to the users' national standards body. A complete listing of these bodies can be found on the CEN website.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and the United Kingdom.

Endorsement notice

The text of ISO 23783-2:2022 has been approved by CEN as EN ISO 23783-2:2023 without any modification.

Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Abbreviated terms	1
5 Measurement methods	2
5.1 Overview of methods suitable for measuring ALHS performance	2
5.2 Photometric methods	9
5.2.1 Dual-dye ratiometric photometric method	9
5.2.2 Single-dye photometric method	9
5.2.3 Fluorescence method	9
5.3 Gravimetric methods	9
5.3.1 Single channel method	9
5.3.2 Regression analysis	10
5.4 Hybrid photometric/gravimetric method	10
5.5 Dimensional methods	10
5.5.1 Optical image analysis of droplets	10
5.5.2 Optical image analysis of capillaries	11
6 Equipment and preparation	11
6.1 Test equipment	11
6.2 Manually operated single- and multi-channel pipettes	12
6.3 Preparation for testing	12
7 Thermal expansion	13
8 Traceability and measuring system uncertainty	13
8.1 Traceability	13
8.2 Estimation of measuring system uncertainty	13
8.2.1 Whole system approach	13
8.2.2 Measurement model approach	13
9 Reporting	14
Annex A (normative) Calculation of liquid volumes from balance readings	15
Annex B (normative) Dual-dye ratiometric photometric procedure	18
Annex C (normative) Single dye photometric procedure	24
Annex D (normative) Gravimetric procedure, single channel measurement	29
Annex E (normative) Gravimetric regression procedure	33
Annex F (normative) Photometric/gravimetric hybrid procedure	39
Annex G (normative) Optical image analysis of droplets	48
Annex H (normative) Fluorescence procedure	57
Annex I (normative) Optical image analysis of capillaries	70
Bibliography	76

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:	— Microplate absorbance reader capable of measuring absorbance at 520 nm and 730 nm;
			384	0,01 to 55,0	2,5 to 5,5	0,35 to 0,55	Aqueous: 15 °C to 30 °C DMSO ^c : 19 °C to 30 °C RH ^d : 20 % to 90 %	— 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.
^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;
			384	0,25 to 20,0	3	1,5		— 96- or 384-well microplates with optically clear bottom; — balance; — magnetic stirrer; — microplate shaker; — pH meter; — manual pipettes; — volumetric flasks.
^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, DMSO ^c	384 1 536	0,001 to 0,015 0,001 to 0,015	<8 <8	<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.
^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	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	— Balance;
				20 to <200	≤0,9	≤0,3	RH ^d : 45 % to 80 %	— density meter;
				200 to 1 000	≤0,9	≤0,3		— anti-electrostatic equipment;
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	— anti-vibration table;
				0,015 to <0,050	2 to 5	2,5 to 5		— temperature- and humidity control for environment;
				0,050 to 1	0,5 to 2	<0,5		— draft shield or draft-free environment for balance.
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;
				0,015 to <0,050	2 to 5	2,5 to 5		— density meter with 6 decimal places;
				0,050 to 1	0,5 to 2	<0,5		— anti-electrostatic equipment;
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	— anti-vibration table;
				0,015 to <0,050	2 to 5	2,5 to 5		— temperature- and humidity control for environment;
				0,050 to 1	0,5 to 2	<0,5		— draft shield or draft-free environment for balance.
^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	Volume range	Typical systematic error ^a	Typical random error ^a	Environmental conditions ^b	Test equipment
			wells	μl	%	%		
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	— 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,
			384	1,0 to 20,0	0,4 to 1,0	0,9 to 1,5	17 °C to 30 °C	— 4-nitrophenol: 405 nm and 620 nm, — Tartrazine: 450 nm and 620 nm,
			96	10 to 1 000 5 to 250	<1 to 5	1 to 2	Temperature stability: < ±0,5 °C	— Tartrazine: 450 nm and 620 nm,
	4-nitrophenol as chromophore	Aqueous	96, 384	1 to 60 0,5 to 25	<1 to 5	1 to 2	RH ^d : 45 % to 80 % RH ^d stability: < ±10 %	— Orange G: 492 nm and 620 nm
			384, 1 536	0,1 to 8	<2 to 10	2 to 5		— 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.
	Orange Gas chromophore	Aqueous	96	1 to 100	<1 to 5	1,5		
			384	1 to 50	<1 to 5	1,5		
			Typically, larger test volumes lead to smaller errors.					
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.								
Dimethylsulfoxide.								
Relative humidity.								

Table 1 (continued)

Method Ref.	Method	Liquid type	Plate type	Volume range	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 $V < 0,5 \mu\text{l}$	<5	<2	Temperature: $(20 \pm 3) ^\circ\text{C}$ or $(27 \pm 3) ^\circ\text{C}$ 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 >1,0 to 1 000	<10 <5	<7 <4	Temperature: 15 °C to 35 °C RH ^d : 15 % to 90 %	— Flatbed scanner; — image analysis software; — specialized plates with capillaries.
^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);

— monitoring of the barometric pressure, which affects the mass to volume conversion.

Traceability of the measurement results to the SI is achieved through the use of a calibrated balance and accounting for test liquid density and air buoyancy.

The procedure for the single-channel gravimetric method specified in [Annex D](#) shall be followed.

5.3.2 Regression analysis

The gravimetric regression method (GRM) is suitable for the measurement of very small liquid volumes, between 0,005 µl and 1 µl. The method is based on a gravimetric balance as the primary measurement device.

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

The key difference to traditional gravimetric methods used for the measurement of larger volumes is the determination of the target volume: a series of balance readings is recorded over a period of time before and after the device under test has delivered the liquid to be measured into the receptacle on the balance. The measurement result of the delivered test liquid is then determined as the difference between two linear regression lines fitted to the recorded balance data before and after the liquid delivery. This method allows measurement of balance drift due to evaporation and other disturbances of the measurement (e.g. by vibrations during the data acquisition), so that these can be compensated for in the measurement calculation (see Reference [6] for more details).

Accommodations regarding the placement of the balance and environmental control and monitoring given in [5.3.1](#) shall be made.

Traceability of the measurement results to the SI is achieved through the use of a calibrated balance.

The procedure for the gravimetric regression method specified in [Annex E](#) shall be followed.

5.4 Hybrid photometric/gravimetric method

The photometric / gravimetric hybrid method allows the evaluation of volumetric performance of ALHS by a combination of a gravimetric measurement with subsequent photometric measurements. Test liquid containing a chromophore is delivered into 96-well or 384-well microplates. The systematic error is determined by gravimetry of the aggregate deliveries into the microplate. Subsequently, the random error of volume deliveries is determined photometrically by measuring the relative absorbances of each well of the microplate.

Chromophores suitable for this method are Tartrazine, Orange G, and 4-nitrophenol. The procedure described in [Annex F](#) is suitable for test volumes between 1 µl and 200 µl in 96-well plates, and 1 µl and 50 µl in 384-well plates.

Accommodations regarding the placement of the balance and environmental control and monitoring given in [5.3.1](#) shall be made.

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

The procedure for the hybrid method specified in [Annex F](#) shall be followed.

5.5 Dimensional methods

5.5.1 Optical image analysis of droplets

This method measures the volume of delivered liquids by analysing images acquired by a high-speed camera and stroboscopic illumination during the liquid delivery cycle. It is suitable for ALHS.

which deliver liquid volumes as a sequence of discrete micro droplets (for further information on the determination of droplet volumes, see Reference [7]).

Traceability of the measurement results to the SI is achieved through calibration of the length scale of the acquired images.

The procedure for the optical image analysis of droplets specified in [Annex G](#) shall be followed.

5.5.2 Optical image analysis of capillaries

The method is based on the optical analysis of images acquired of capillaries of known and calibrated geometry. For the image acquisition, a flatbed scanner is used. The method provides a direct determination of the volume by the optical measurement of one or multiple lengths of the calibrated capillaries.

The method can be used to measure volumes between 0,1 µl and 1 000 µl for ALHS with 1 to 384 channels. The measurement uncertainties only minimally depend on the environmental conditions and are reliable in a broad range of environmental conditions without error corrections.

Traceability of the measurement results to the SI is achieved by using calibrated capillaries and a calibrated image acquisition device.

The procedure for the optical image analysis of capillaries specified in [Annex I](#) shall be followed.

6 Equipment and preparation

6.1 Test equipment

Test equipment used for volumetric performance measurements according to the procedures described in this document shall conform to the minimum performance requirements given in [Tables 2, 3, 4, and 5](#), unless different minimum performance requirements are described within a specific test procedure. Balances shall be allowed to settle for at least 6 s before reading the indicated value.

NOTE 1 The balance repeatability and expanded uncertainty in use given in [Table 2](#) are harmonized with [Table 3](#) but the weighing ranges are restricted to improve accuracy when weighing an amount of dry chemicals.

NOTE 2 The balance requirements given in [Table 3](#) are based on ISO 8655-6 and allow multiple replicate deliveries of test liquid into the same weighing vessel without emptying it out.

Table 2 — Minimum requirements for balances for weighing dry materials

Smallest amount to be weighed g	Readability mg	Repeatability mg	Expanded uncertainty in use ^a ($k = 2$) mg
<1,0	0,001	0,006	0,012
1,0	0,01	0,025	0,05
10	0,1	0,2	0,4
100	1	2	4
1 000	10	20	40

^a Uncertainty in use can be determined according to ASTM E898-20[8] and EURAMET CG-18[9] at the minimum mass listed in the table.

Table 3 — Minimum requirements for balances for weighing liquids

Delivered volume of test liquid ^a μl	Readability mg	Repeatability mg	Expanded uncertainty in use ^b ($k = 2$) mg
<0,5	0,000 1	0,000 5	0,001
$0,5 \leq V < 20$	0,001	0,006	0,012
$20 \leq V < 200$	0,01	0,025	0,05
$200 \leq V \leq 10\,000$	0,1	0,2	0,4

^a Assumes one delivery of test liquid from a single channel.

^b Uncertainty in use can be determined according to ASTM E898-20^[8] and EURAMET CG-18^[9] at the value of the largest volume of the listed range, assuming a single channel delivery.

Table 4 — Minimum performance requirements for absorbance microplate readers

Parameter	Requirement
Photometric resolution	0,001 AU ^b
Photometric trueness ^a from 0 AU to 1,0 AU	0,005 AU
Photometric trueness ^a from > 1,0 AU to 2,0 AU	0,010 AU
Photometric repeatability from 0 AU to 2,0 AU	0,005 AU
System linearity between 0 AU and 2,0 AU	0,010 AU
Wavelength accuracy	< ± 1,5 nm

^a Photometric trueness is sometimes called photometric accuracy.

^b Absorbance unit.

Table 5 — Minimum performance requirements for other test equipment

Instrument	Resolution	Expanded uncertainty ($k = 2$)
Thermometer for liquids	0,1 °C	0,2 °C
Thermometer for ambient air	0,1 °C	0,2 °C
Hygrometer	1 % RH ^a	5 % RH ^a
Barometer	0,1 kPa	1 kPa
Timing device	1 s	not relevant

^a Relative humidity.

6.2 Manually operated single- and multi-channel pipettes

Single-channel and multi-channel pipettes shall be calibrated and shall fulfil the minimum performance requirements given in ISO 8655-2. Operator impact on manually pipetted volumes shall be considered in the calculation of errors and measurement uncertainty.

6.3 Preparation for testing

The ALHS under test, including all exchangeable parts to be used during the test, and all test equipment and test liquids shall be in thermal equilibrium (± 2 °C) for at least 2 h prior to the start of testing. During the time of testing, the environmental conditions shall not change more than ± 1 °C and 5 % relative humidity (RH). Requirements for environmental conditions are described in each test procedure. Laboratory instrumentation is designed to be operated under non-condensing humidity conditions.

All test equipment shall be calibrated according to the test procedure in this document, or according to the manufacturer's instructions if the calibration is not specifically explained in the procedure.

The ALHS under test and test equipment shall be powered on and allowed sufficient time to equilibrate for proper functionality according to the manufacturer's instructions.

Liquid reservoirs of ALHS shall be filled immediately before testing begins. ALHS which require priming shall be primed according to the manufacturer's instructions immediately before testing begins.

The tips of piston-operated ALHS should be pre-rinsed with the test liquid at least five times, whereby the dispensed test liquid is discarded to waste. This step is required every time a tip is changed.

7 Thermal expansion

If the test temperature is different from the temperature of adjustment of the ALHS, and if the cubic thermal expansion coefficient γ of the volumetric apparatus is known, [Formula \(1\)](#) may be used to correct the measured volume of test liquid for thermal expansion:

$$V_{L,tc} = V_L \times [1 - \gamma \times (t_T - t_{ref})] \quad (1)$$

where

$V_{L,tc}$ is the delivered volume of test liquid, corrected for thermal expansion of the ALHS;

V_L is the delivered volume of test liquid measured at the test temperature;

γ is the cubic thermal expansion coefficient of the volumetric apparatus;

t_T is the temperature at which the test is performed;

t_{ref} is the reference temperature of adjustment of the ALHS.

8 Traceability and measuring system uncertainty

8.1 Traceability

All measurements by relevant test equipment used for the calibration of an ALHS shall be traceable to the International System of Units (SI). The uncertainty of relevant test equipment affects the error of the reported volumetric results.

NOTE Relevant test equipment can include but is not limited to: balances, thermometers, pH meters, hygrometers, plate readers, spectrophotometers, calibrated pipettes, volumetric glassware, size calibration charts, and timing devices.

8.2 Estimation of measuring system uncertainty

8.2.1 Whole system approach

Measuring system uncertainty (MSU) may be estimated by statistical evaluation of results produced by the entire measuring system. Precision and bias studies, measurement system analysis, and inter-laboratory comparisons are some of the means by which this is achieved. Detailed approaches are described in Reference [\[4\]](#).

8.2.2 Measurement model approach

This approach estimates MSU based on an analysis of each input to a measurement model. This approach is detailed in Reference [\[3\]](#).

9 Reporting

Measurement results, traceability, and measurement uncertainty shall be reported in accordance with ISO 23783-3.

Annex A (normative)

Calculation of liquid volumes from balance readings

A.1 Calculation of liquid volume from the balance reading

A.1.1 General formula for volume

For the conversion of the balance reading of the mass, m , to volume, V , at the test temperature, a correction for the liquid's density and air buoyancy is necessary. The calculation of the liquid volume at the test temperature is given by [Formula \(A.1\)](#).

In case the test liquid is water and the calculations given in [Clause A.1](#) are not feasible, the balance indications may be converted to volume using the correction factors given in [Clause A.2](#).

NOTE 1 [Formula \(A.1\)](#) is based on Formula (1) of ISO 4787:2021, however, the thermal expansion of the ALHS is neglected and only the thermal expansion of the test liquid is accounted for.

$$V_L = (m_L - m_E) \times \frac{1}{\rho_L - \rho_A} \times \left(1 - \frac{\rho_A}{\rho_B} \right) \quad (\text{A.1})$$

where

V_L is the calculated volume of test liquid at the test temperature, in ml;

m_L is the balance reading of the weighing vessel after test liquid delivery, in g;

m_E is the balance reading of the weighing vessel before test liquid delivery, in g ($m_E = 0$ in case the balance was tared with the weighing vessel);

ρ_A is the density of air, in g/ml, see [Formula \(A.2\)](#);

ρ_B is the actual or assumed density of the weights used to calibrate the balance, in g/ml;

NOTE 2 Stainless steel weights of density 8,0 g/ml are typically used for balance calibration.

ρ_L is the density of the test liquid at the test temperature t (in °C), in g/ml.

For water, ρ_L can be calculated with the "Tanaka" [Formula \(A.3\)](#); the density of other test liquids should be determined with sufficiently small uncertainty (see [A.1.4](#)).

A.1.2 Calculation of air density

[Formula \(A.2\)](#) for air density can be used at temperatures between 15 °C and 27 °C, barometric pressure between 600 hPa and 1 100 hPa, and relative humidity between 20 % and 80 %.

$$\rho_A = \frac{1}{1\,000} \times \frac{0,34\,848 \times p - 0,009 \times h_r \times e^{(0,061 \times t)}}{t + 273,15} \quad (\text{A.2})$$

where

ρ_A is the air density, in g/ml;

- t is the ambient temperature, in °C;
 p is the barometric pressure, in hPa;
 h_r is the relative air humidity, in %.

The uncertainty of this formula can be calculated according to OIML R 111-1:2004, section C.6.3.6 [11]. At other environmental conditions, [Formula \(A.2\)](#) shall be replaced with the current CIPM air density equation.

NOTE The air density calculation valid at the time of publication of this document is CIPM-2007 and is described in Reference [12].

A.1.3 Calculation of water density

The density of pure water is normally calculated using formulae given in the literature. [Formula \(A.3\)](#), as published by Tanaka[13] provides a good basis for standardization:

$$\rho_W = a_5 \times \left[1 - \frac{(t_W + a_1)^2 \times (t_W + a_2)}{a_3 \times (t_W + a_4)} \right] \quad (\text{A.3})$$

where

- ρ_W is the density of water, in g/ml
 t_W is the water temperature, in °C
 $a_1 = -3,983\,035\,^{\circ}\text{C}$
 $a_2 = 301,797\,^{\circ}\text{C}$
 $a_3 = 522\,528,9\,(^{\circ}\text{C})^2$
 $a_4 = 69,348\,81\,^{\circ}\text{C}$
 $a_5 = 0,999\,974\,950\,\text{g/ml}$

A.1.4 Test liquids other than water

When the test liquid is not pure water, the density of the test liquid ρ_L cannot be calculated by [Formula \(A.3\)](#). For these test liquids, density shall be measured or obtained from literature values.

NOTE The error associated with density measurements or use of literature values can range from negligible to significant.

A.2 Correction factors for the conversion of balance readings to volume

If the test liquid is distilled water, another possible procedure for the conversion of mass to volume is using the Z correction factors specified in [Table A.1](#).

Convert each mass m_i obtained from balance readings by applying the Z correction factors at the mean temperature and barometric pressure measured during the measurement procedure, and using [Formula \(A.4\)](#):

$$V_i = m_i \times Z \quad (\text{A.4})$$

where

V_i is the delivered test volume, in μl ;

m_i is the measured mass of the delivered test volume, in mg;

Z is the Z correction factor from [Table A.1](#), in $\mu\text{l}/\text{mg}$.

Table A.1 — Z correction factors for distilled water (air-saturated) in units of μl per mg

Temperature °C	Barometric pressure						
	hPa						
	800	850	900	950	1 000	1 013	1 050
15,0	1,001 7	1,001 8	1,001 9	1,001 9	1,002 0	1,002 0	1,002 0
15,5	1,001 8	1,001 9	1,001 9	1,002 0	1,002 0	1,002 0	1,002 1
16,0	1,001 9	1,002 0	1,002 0	1,002 1	1,002 1	1,002 1	1,002 2
16,5	1,002 0	1,002 0	1,002 1	1,002 1	1,002 2	1,002 2	1,002 2
17,0	1,002 1	1,002 1	1,002 2	1,002 2	1,002 3	1,002 3	1,002 3
17,5	1,002 2	1,002 2	1,002 3	1,002 3	1,002 4	1,002 4	1,002 4
18,0	1,002 2	1,002 3	1,002 3	1,002 4	1,002 5	1,002 5	1,002 5
18,5	1,002 3	1,002 4	1,002 4	1,002 5	1,002 5	1,002 6	1,002 6
19,0	1,002 4	1,002 5	1,002 5	1,002 6	1,002 6	1,002 7	1,002 7
19,5	1,002 5	1,002 6	1,002 6	1,002 7	1,002 7	1,002 8	1,002 8
20,0	1,002 6	1,002 7	1,002 7	1,002 8	1,002 8	1,002 9	1,002 9
20,5	1,002 7	1,002 8	1,002 8	1,002 9	1,002 9	1,003 0	1,003 0
21,0	1,002 8	1,002 9	1,002 9	1,003 0	1,003 1	1,003 1	1,003 1
21,5	1,003 0	1,003 0	1,003 1	1,003 1	1,003 2	1,003 2	1,003 2
22,0	1,003 1	1,003 1	1,003 2	1,003 2	1,003 3	1,003 3	1,003 3
22,5	1,003 2	1,003 2	1,003 3	1,003 3	1,003 4	1,003 4	1,003 4
23,0	1,003 3	1,003 3	1,003 4	1,003 4	1,003 5	1,003 5	1,003 6
23,5	1,003 4	1,003 5	1,003 5	1,003 6	1,003 6	1,003 6	1,003 7
24,0	1,003 5	1,003 6	1,003 6	1,003 7	1,003 7	1,003 8	1,003 8
24,5	1,003 7	1,003 7	1,003 8	1,003 8	1,003 9	1,003 9	1,003 9
25,0	1,003 8	1,003 8	1,003 9	1,003 9	1,004 0	1,004 0	1,004 0
25,5	1,003 9	1,004 0	1,004 0	1,004 1	1,004 1	1,004 1	1,004 2
26,0	1,004 0	1,004 1	1,004 1	1,004 2	1,004 2	1,004 3	1,004 3
26,5	1,004 2	1,004 2	1,004 3	1,004 3	1,004 4	1,004 4	1,004 4
27,0	1,004 3	1,004 4	1,004 4	1,004 5	1,004 5	1,004 5	1,004 6
27,5	1,004 5	1,004 5	1,004 6	1,004 6	1,004 7	1,004 7	1,004 7
28,0	1,004 6	1,004 6	1,004 7	1,004 7	1,004 8	1,004 8	1,004 8
28,5	1,004 7	1,004 8	1,004 8	1,004 9	1,004 9	1,005 0	1,005 0
29,0	1,004 9	1,004 9	1,005 0	1,005 0	1,005 1	1,005 1	1,005 1
29,5	1,005 0	1,005 1	1,005 1	1,005 2	1,005 2	1,005 2	1,005 3
30,0	1,005 2	1,005 2	1,005 3	1,005 3	1,005 4	1,005 4	1,005 4

Annex B (normative)

Dual-dye ratiometric photometric procedure

B.1 General

The delivered volumes into wells of microplates are determined using two chromophore solutions with known concentrations of each chromophore. Ponceau S and copper(II) chloride have absorbance maxima at 520 nm and 730 nm, respectively. All liquids contain the same concentration of copper(II) chloride, which is used to determine the fill height of each well. The test volume of Ponceau S test liquid is delivered into each well, mixed, and absorbances are read at 520 nm and 730 nm to calculate the delivered test liquid volume.

B.2 Test equipment

B.2.1 General

All test equipment shall be chosen such that the required measuring system uncertainty (MSU) can be obtained. The MSU can be estimated according to the approach given in ISO/IEC Guide 98-3.

B.2.2 Microplate absorbance reader

A microplate absorbance reader meeting the minimum performance requirements according to [Table 4](#) shall be used. Bandpass filters with centre wavelengths of (520 ± 1) nm and (730 ± 2) nm shall be installed in the plate reader. Alternatively, plate readers with monochromators suitable to select (520 ± 1) nm and (730 ± 2) nm as centre wavelengths may be used.

B.2.3 Spectrophotometer

Absorbance values of the copper(II) chloride and Ponceau S liquids shall be measured on a spectrophotometer with the minimum performance specifications as listed in [Table B.1](#). Bandpass filters with centre wavelengths of (520 ± 1) nm and (730 ± 2) nm shall be installed in the spectrophotometer.

A calibrated cuvette with an optical path length of 10 mm shall be used for the absorbance measurements.

Table B.1 — Minimum performance requirements of the spectrophotometer

Parameter	Requirement
Photometric resolution	0,000 1 AU ^c
Photometric repeatability at A = 0,0 AU ^a	0,000 15 AU
Photometric repeatability at A = 0,5 AU ^a	0,000 15 AU
Photometric repeatability at A = 1,0 AU ^a	0,000 15 AU
Photometric repeatability at A = 1,5 AU ^a	0,000 15 AU
System linearity between 0 AU and 1,5 AU ^b	0,000 25 AU
^a Bandpass and signal averaging time may be increased to meet the photometric repeatability requirement. ^b Corrections may be applied to meet the system linearity requirement. ^c Absorbance unit.	