INTERNATIONAL STANDARD

ISO 23783-3

First edition
2022-08

Automated liquid handling systems —

Part 3: Determination, specification and reporting of volumetric performance

Systèmes automatisés de manipulation de liquides —

Partie 3: Détermination, spécification et compte-rendu des performances volumétriques

Reference number
ISO 23783-3:2022(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).

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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 23783-3, together with ISO 23783-1 and ISO 23783-2, 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 3:
Determination, specification and reporting of volumetric performance

1 Scope

This document provides guidance and establishes requirements for collecting and examining volumetric performance data of automated liquid handling systems (ALHS). It specifies how to index and track volumetric performance data and provides descriptive statistics for the evaluation of these data. This document also specifies reporting requirements of ALHS volumetric performance.

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. Measurement procedures for the determination of volumetric performance are given in ISO 23783-2.

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

ISO 23783-2:2022, *Automated liquid handling systems — Part 2: Measurement procedures for the determination 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 Volumetric performance

5.1 General

Automated liquid handling systems (ALHS) are designed to deliver amounts of liquid at a target volume. The target volume is typically set using software or other digital control. Volumetric performance shall be assessed by measuring the volume of each liquid delivery and evaluating the data.

Volumetric performance is typically assessed as part of the manufacturing process quality control. Subsequent volumetric performance assessments can be done by suppliers, users, as well as by third-party testing and calibration service providers.

Automated liquid handling systems are designed to handle a variety of liquids of differing physical properties such as density, viscosity, surface tension and contact angle against solid surfaces. Test liquids can be aqueous or other solvents. Aqueous test liquids can be pure water or contain other compounds such as acids, bases, salts, dyes, or other inorganic, organic, or biological compounds. The chemical composition of the test liquid can vary significantly depending on the method and should reflect the liquid used by the ALHS as closely as possible. Since the volumetric performance of the ALHS can vary depending on these physical properties, a description of the test liquid shall be included when reports of volumetric performance are made. This description of the test liquid may be made in terms of chemical composition, physical properties, or both.

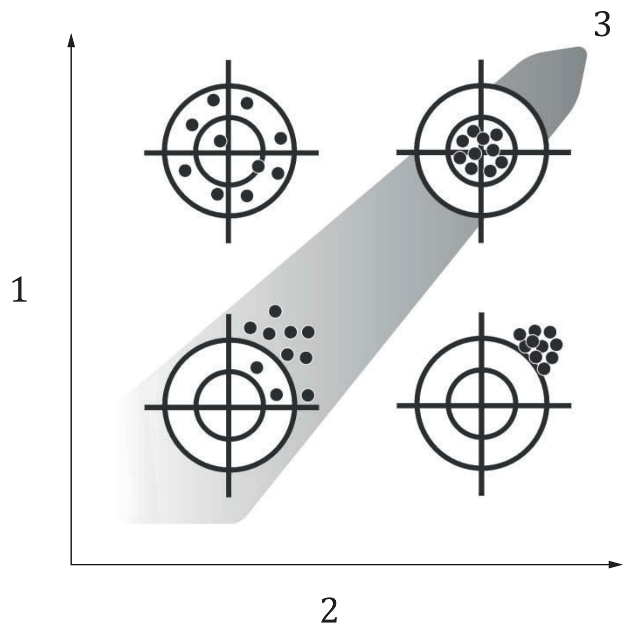
ALHS shall be supplied with performance claims at various volumes for a particular instrument configuration. The maximum specified volume and minimum specified volume establish a liquid handling range for which established volumetric performance specifications are available. The maximum specified volume and minimum specified volume can vary depending on instrument configuration (e.g. disposable tip size, syringe size).

NOTE In preparing for a volumetric performance test, the ALHS will be set to deliver a particular target volume. During testing, each delivered volume is expected to be slightly different from the target volume. The delivered volume is a conceptual quantity because it cannot be known with certainty and can only be approximated by measurement.

In order to evaluate volumetric performance, measurements are made of individual delivered volumes. The measured volume differs slightly from the true delivered volume due to measurement system error, which can be expressed as measuring system uncertainty (MSU). This value should be expressed in accordance with ISO/IEC Guide 98-3.

Accuracy of the ALHS can be improved by improving precision and trueness. These concepts and their inter-dependencies are illustrated in Figure 1. Improving precision brings the cluster of the results into a smaller bunch, while improved trueness occurs when the centre of the cluster is closer to the centre of the target.

Accuracy, precision and trueness are conceptual terms. Quantitative expressions of these concepts are given in terms of error, random error and systematic error, respectively.



Key
1 improving trueness, decreasing systematic errors
2 improving precision, decreasing random errors
3 improving accuracy, decreasing both systematic and random errors

Figure 1 — Relationship between trueness, precision, and accuracy of an ALHS

5.2 Data collection and examination

ALHS test results shall include data sets of individual measured volumes, and descriptive statistics which summarize the data sets. Systematic error and random error are two examples of descriptive statistics which are commonly employed in the testing of ALHS.

Each instance of a delivered volume shall be measured to determine a measured volume, V. Measured volumes shall be determined in accordance with one of the measurement methods described in ISO 23783-2.

Prior to calculating descriptive statistics, it is recommended that the measured volumes be visually examined for evidence of outliers, trending, or patterns. Such features can indicate the need for more detailed analysis, optimization, or additional testing to determine the cause. For the purposes of this document, outliers are considered to be unusual results that cannot be reliably repeated. Trending refers to results that vary in a regular way when viewed by time or dispense order. It is possible to observe patterns when viewing data in a spatial arrangement such as by examining results distributed in a plate arrangement. Visualization aids such as heat mapping may be used to help identify patterns. The presence of outliers, trending or patterns can indicate the need for further investigation, including optimization or repair of the ALHS.

NOTE 1 Statistical consideration of outliers is beyond the scope of this document.

NOTE 2 The formulae presented in this document are sufficient to describe volumetric data that are normally distributed. When data are not normally distributed, it can be necessary to provide additional information to adequately describe ALHS performance (see Reference [2] for more details). For example, ALHS which exhibit trending, as well as multi-dispensing modes are two cases where volumetric data are not normally distributed.

5.3 Indexing to track data

5.3.1 General

With multiple different channels, replicates and experimental possibilities, an identification scheme is needed to keep track of the data.

NOTE Additional explanations and examples of the indexing scheme can be found in Reference [3].

5.3.2 Indexing from the channel perspective

Viewed from the perspective of the liquid handler, each volume delivery can be given an index number in the form of an ordered triplet of integers (l,m,n) where:

- l is an index for the dispensing channel and the value ranges from 1 to L. The variable L is the number of dispensing channels per ALHS. L can be as small as 1 for the case of a single channel device, to 384 or greater.
- m is an index for a reproducible experiment, and the value ranges from 1 to M. The variable M is used to track different experiments under different reproducibility conditions. For example, replicates of prior experiments when assessing reproducibility or drift over longer time periods.
- n is an index for delivery order within a single repeatability test (run) and the value ranges from 1 to N. The variable N is the number of replicates in a repeatability test (run) where the volumes are delivered in a short period of time under nearly identical repeatability conditions. One repeatability test can require the use of multiple microplates.

In this way, a measured volume V of the n-th delivery by the l-th channel, during the m-th experiment is given by the symbol $V(l,m,n)$.

This document does not specify a minimum number of replicates for routine testing, and a minimum of 10 replicates for calibration of ALHS (see ISO 23783-1:2022, 6.5). The number of replicates (N) shall be reported when repeatability data are used to calculate averages or standard deviations as the reliability of these descriptive statistics depends on the number of replicates.

The channel perspective is recommended for purposes of evaluating volumetric performance and determining whether particular channels are performing correctly. Alternative indexing systems such as the microplate perspective are described in 5.3.3. Examples illustrating these systems are provided in Annex A.

5.3.3 Indexing from the microplate perspective

When volumes are dispensed into microplates for measurement, it is common to index by row, column, and plate. In 96- and 384-well microplates, it is common for rows to be designated by letters (e.g. A through H, and A through P, respectively) while columns are numbered (1 through 12, and 1 through 24, respectively). This viewpoint is recommended for evaluating precision, trueness, or accuracy from a plate perspective. Indexing schemes are not mutually exclusive. When volume measurements are made in microplates, knowledge of the liquid handling system programming allows the data from the rows, columns and plates to be translated into the channel, run, and dispense order.

It is not necessary to consider different plates to be different experiments. For example, a 96-tip head could be tested by making a series of deliveries into three 96-well plates. In this case, plates 1, 2 and 3 can be considered to be dispense replicates n = 1, 2 and 3, while all three plates are considered part of a single experiment.

NOTE 1 An example of the above-mentioned scenario is included in A.5.

NOTE 2 Frequently there is interest in "within plate" variation or variation and patterns across different plates. For example, when patterns are observed within a plate, it can be of interest whether the pattern is repeatable across additional plates. Also, when evaluating different ALHS for a particular application, it can be useful to evaluate data from the plate perspective without regard to the arrangements of independent channels and thus simply compare the whole plate precision of two different systems.

5.4 Descriptive statistics on an individual channel basis

5.4.1 General

An ALHS typically performs a series of *N* replicate deliveries of the target volume, which are averaged to calculate the actual delivered volume. These *N* replicates are usually delivered within a short period of time under repeatability conditions and are referred to as a "run." The ALHS can be programmed so that a run is preceded by one or more pre-deliveries of test liquid. Pre-deliveries should be performed after a period of ALHS inactivity or changes to the ALHS parameters (e.g. target volume, liquid class, test liquid), and should be delivered into the waste. The reproducibility between experiments (*M*) is increased if each run is started under similar, well-defined test conditions.

5.4.2 Average volume

The average volume delivered by a particular channel during a particular run is given in Formula (1). This average volume can then be used to calculate both systematic and random errors.

$$\bar{V}(l,m)=\frac{1}{N}\times\sum_{n=1}^{N}V(l,m,n) \tag{1}$$

where

$\bar{V}(l,m)$ is the average of all *N* measured volumes from channel '*l*' during experiment '*m*';

N is the number of replicate deliveries in the run;

$V(l,m,n)$ is a single measured volume.

5.4.3 Systematic error

Systematic error is estimated by the deviation of the measured mean volume from the target volume. If the ALHS is set to deliver a target volume of V_T = 100 µl, and then delivers an actual volume of 97 µl, the systematic error is −3 µl (absolute error) or −3 % (relative error). The determination of the systematic error of a single channel in a single run is given by Formula (2). Formula (2) can be generalized and applied in any situation where it is desired to compare a measurement result to the target volume.

The systematic error in the ISO 23783 series is based on historic convention within the pipetting industry and is reversed in sign compared to ISO/IEC Guide 99:2007, 2.17 because in the ISO 23783 series, the target volume is considered the reference value.

$$e_S(l,m)=\bar{V}(l,m)-V_T \tag{2}$$

where

$e_S(l,m)$ is the systematic error of channel '*l*' during experiment '*m*' expressed in units of volume;

V_T is the target volume, the volume intended to be delivered.