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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

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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).

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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.

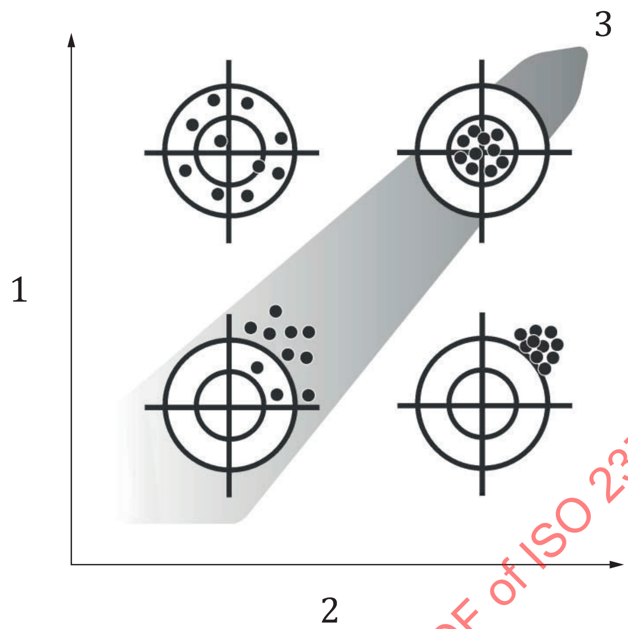
ALHS shall be supplied with performance claims at various volumes for a particular instrument configuration. The maximum specified volume and minimum specified volume establish a liquid handling range for which established volumetric performance specifications are available. The maximum specified volume 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.

The systematic error can also be expressed in relative terms as shown in Formula (3):

$$\eta_S(l,m)=\frac{\bar{V}(l,m)-V_T}{V_T}\times 100\,\% \tag{3}$$

where $\eta_S(l,m)$ is the relative systematic error of channel '*l*' during experiment '*m*.'

Estimates of systematic error can be improved by increasing the number of measurements in the data set, either by increasing *N*, or conducting multiple reproducible experiments and summing over both *N* and *M.* Increasing *N* is accommodated in Formula (1), while summing over multiple experiments is shown in Formula (4).

$$\bar{V}(l)=\frac{1}{M\times N}\times\sum_{m=1}^{M}\sum_{n=1}^{N}V(l,m,n) \tag{4}$$

where

$\bar{V}(l)$ is the mean measured volume from channel '*l*';

M is the number of experiments included in the average.

Formula (4) can be re-arranged as shown in Formula (5), and the identical result can be obtained by either Formula (4), or by taking the *M* reproducible results of Formula (1), and averaging them together as shown in Formula (5).

$$\bar{V}(l)=\frac{1}{M}\times\sum_{m=1}^{M}\frac{1}{N}\sum_{n=1}^{N}V(l,m,n)=\frac{1}{M}\times\sum_{m=1}^{M}\bar{V}(l,m) \tag{5}$$

5.4.4 Random error

The random error of a channel is usually assessed by calculating the standard deviation of a series of *N* measured volumes under repeatability conditions, as shown in Formula (6).

$$s_r(l,m)=\sqrt{\frac{\sum_{n=1}^{N}\left[V(l,m,n)-\bar{V}(l,m)\right]^2}{N-1}} \tag{6}$$

where $s_r(l,m)$ is the random error of channel '*l*' during experiment '*m*.'

This standard deviation can be divided by the average volume and multiplied by 100 to convert to a percentage as shown in Formula (7). This is the recommended descriptive statistic for random error and is often called the coefficient of variation (CV).

$$C_V(l,m)=\frac{s_r(l,m)}{\bar{V}(l,m)}\times 100\,\% \tag{7}$$

where $C_V(l,m)$ is the coefficient of variation of channel '*l*' during experiment '*m*.'

Estimates of random error can also be improved by increasing the number of measurements in the data set, either by increasing *N*, or by conducting multiple reproducible experiments and combining the results. Increasing *N* is accommodated in Formula (6).

Averaging CV over multiple experiments can be accomplished using a root-mean-squares approach as shown in Formula (8). Formula (8) may be used when N is identical in each experiment.

$$C_V(l)=\sqrt{\frac{\sum_{m=1}^{M}[C_V(l,m)]^2}{M}} \tag{8}$$

where $C_V(l)$ is the coefficient of variation of channel 'l' combining data from multiple experiments.

CV results should not be combined by simple averaging (arithmetic mean).

When combining or averaging random errors, the precise details of the mathematical operation matter, and depending on the details, can lead to a different value of the CV result. If a formula other than Formula (8) is used, the formulae used shall be described in sufficient detail to permit an unambiguous understanding [see 6.1.2 p)].

Examples applying these channel statistics are included in Annex A. Evaluation based on channel statistics is frequently used to determine whether channels are working properly.

5.5 Descriptive statistics on a run order basis

In some cases, it is useful to view data on a run order basis. While channel analysis is useful for determining whether an instrument requires repair or maintenance, run order analysis can be particularly valuable during method development to determine whether the liquid handling protocol is properly optimized to prevent systematic trending effects during the liquid delivery sequence. For example, some programming choices can result in a "first shot effect" where the n = 1 delivery is consistently greater or lesser than subsequent deliveries.

The mean volume on a run order basis $\bar{V}(n)$ can be calculated by Formula (9).

$$\bar{V}(n)=\frac{1}{L\times M}\times\sum_{l=1}^{L}\sum_{m=1}^{M}V(l,m,n) \tag{9}$$

where

- $\bar{V}(n)$ is the mean measured volume from the 'n'-th dispense of all channels and all experiments;
- L is the number of channels in the ALHS.

NOTE The case where only one experiment is performed (M = 1) can be accommodated within Formula (9) and Formula (10).

In addition to run-order volume, a run-order random error can be calculated using Formula (10) and expressed as a CV using Formula (11). These statistics can be useful in determining whether the random error or CV changes during the dispense order. For example, when a disposable pipette tip is re-used a number of times, the CV can eventually increase.

$$s_r(n)=\sqrt{\frac{\sum_{l=1}^{L}\sum_{m=1}^{M}\left[V(l,m,n)-\bar{V}(n)\right]^2}{L\times M-1}} \tag{10}$$

where $s_r(n)$ is the standard deviation of the n-th dispense across all channels, and combining data from multiple experiments.

$$C_V(n)=\frac{s_r(n)}{\bar{V}(n)}\times 100\,\% \tag{11}$$

where $C_V(n)$ is the coefficient of variation of the n-th dispense across all channels, and combining data from multiple experiments.

5.6 Descriptive statistics for entire data sets

The grand average volume is useful in determining the overall trueness at a particular target volume; it is the arithmetic mean of all measured volumes in the data set, and calculated using Formula (12). The grand average volume can be converted to a systematic error by analogy to Formula (2) and relative systematic error by analogy to Formula (3).

$$\bar{V}_{\mathrm{GA}}=\frac{1}{L\times M\times N}\times\sum_{l=1}^{L}\sum_{m=1}^{M}\sum_{n=1}^{N}V(l,m,n) \tag{12}$$

where $\bar{V}_{\mathrm{GA}}$ is the grand average volume calculated from all channels, all replicates, and all experiments.

Overall CV ($C_{V,\mathrm{OA}}$) includes contributions from random error within each individual channel, and also contributions from systematic differences between channels. In some cases, the overall CV is of particular interest and can be calculated using Formula (13).

$$C_{V,\mathrm{OA}}=\frac{\sqrt{\frac{1}{(L\times M\times N-1)}\times\sum_{l=1}^{L}\sum_{m=1}^{M}\sum_{n=1}^{N}\left[V(l,m,n)-\bar{V}_{\mathrm{GA}}\right]^2}}{\bar{V}_{\mathrm{GA}}}\times 100\,\% \tag{13}$$

where $C_{V,\mathrm{OA}}$ is the overall coefficient of variation of all measurements within the data set.

5.7 Differences between channels

Systematic differences between channels are of concern when it is intended to limit or compensate for variation in experimental results, and also when it is intended to improve overall CV in a system.

One way to limit differences between channels is to establish limits for systematic error and apply them to each channel. Therefore, when establishing or evaluating specifications for systematic error it is important to consider whether the limits apply to each channel evaluated individually, or only to the systematic error calculated from the grand average volume.

Channel-to-channel differences can also be quantified as a channel-to-channel CV, calculated as in Formula (14).

$$C_{V,\mathrm{C2C}} = \frac{\sqrt{\frac{1}{(L-1)} \times \sum_{l=1}^{L} \left[\bar{V}(l) - \bar{V}_{\mathrm{GA}}\right]^2}}{\bar{V}_{\mathrm{GA}}} \times 100\,\% \tag{14}$$

where $C_{V,\mathrm{C2C}}$ is the coefficient of variation of the mean volumes delivered by each channel.

The channel-to-channel CV need not be specified or calculated in all cases. However, it can be useful in determining whether channel-to-channel CV is a significant contributor to the overall CV.

5.8 Volume increments

Some systems deliver liquids by depositing multiple sub-deliveries to form a final delivered volume. For example, acoustic dispensers, ink jet type dispensers, and some other systems can deliver in this way. For the purposes of this document, assessing the volumetric performance of such systems is based on the volume of the combined deliveries, without individual measurement of each sub-delivery.

6 Reporting

6.1 Reporting of the results

6.1.1 General

The results of each test, calibration, or series of tests or calibrations carried out shall be reported accurately, clearly, unambiguously and objectively, and in accordance with any specific instructions in the test or calibration methods. The results shall be reported, usually in a test report or a calibration certificate, and shall include all of the information requested, necessary for the interpretation of the test or calibration results, and all information required by the specific test method used.

The naming convention of reports and certificates is dependent on regional, national, or local guidelines.

NOTE Test reports and calibration certificates are sometimes called test certificates and calibration reports, respectively.

The test reports or calibration certificates may be issued as hard copies or by electronic means.

6.1.2 Test reports and calibration certificates

At least the following information shall be reported:

a) a title (e.g. “Test Report” or “Calibration Certificate”);

b) the name and address of the site of testing;

c) the date(s) of the test or calibration;

d) the name(s) of the person(s) performing the test;

e) a unique identification of the test report or calibration certificate, and on each page an identification in order to ensure that the page is recognized as a part of the calibration certificate, and a clear identification of the end of the calibration certificate;

f) a description, and unambiguous identification, of the ALHS tested or calibrated, including (when applicable) serial numbers of its components, system configuration, firmware and software versions, liquid class details, and any other detail necessary to reproduce the test of the complete ALHS;

g) a list, and identification (e.g. make, model, and serial or lot number), of all automatically exchangeable components (see ISO 23783-1:2022, 6.8.1) used during the test, and how many automatic exchanges were performed for each of these components during the test;

h) a list, and identification (e.g. make, model, and serial or lot number), of all manually exchangeable components (see ISO 23783-1:2022, 6.8.2) used during the test, and how many manual exchanges were performed for each of these components during the test;

i) a list, and identification (e.g. make, model, and serial or lot number), of all other exchangeable components, which affect the volumetric performance of the ALHS (see ISO 23783-1:2022, 6.8.3), and which have been exchanged prior to the test;

j) the test procedure used and reference to that test procedure according to ISO 23783-2 (example: test was performed according to ISO 23783-2:2022, Annex B);

k) identification of the test liquid(s) used;

l) deviations from, additions to, or exclusions from the referenced test procedure;

m) reference to the test plan and test procedure used, including:

   1) number of channels tested (L),

   2) number of replicate volume dispenses (N),

   3) dispense order into the plate,

   4) parameters l, m, n as described in this document,

   5) all pertinent ALHS settings such as

      i) liquid class,

      ii) aspirate and dispense parameters;

n) number of experiments (M) performed, and a description of the changes in the reproducibility conditions;

o) information on test conditions, such as environmental conditions and other crucial parameters, which have an impact on liquid delivery results;

p) the test or calibration results, including the units of measurement, and any deviations from the formulae described in this document;

q) a record of measuring instruments, reagents, and supplies used in the testing process of the ALHS;

r) where relevant, a statement of compliance/non-compliance with requirements or specifications, including acceptance criteria and units of measurement.

Test reports and calibration certificates should include the page number and total number of pages.

When reporting ALHS performance, it is critical to keep all technical information of the test in mind in order to properly understand the test results and how the declared performance was determined. It is therefore recommended that test report includes a statement specifying that the test report or calibration certificate shall not be reproduced except in full, without written approval.

6.1.3 Calibration certificates

In addition to the requirements listed in 6.1.2, calibration certificates shall include the following, unless otherwise specified:

a) Measurement uncertainty of the calibration. This uncertainty shall include contributions from the MSU, the ALHS under test, and any exchangeable components for which the calibration is valid.

The included exchangeable components shall be listed, as well as the number of exchanged parts tested. The expanded measurement uncertainty shall be reported, and the coverage factor *k* shall be stated.

b) Evidence that the measurement results are traceable to the SI unit of volume. The litre and sub-divisions such as microlitre or nanolitre may be used. Evidence of traceability includes documentation of the calibration status of the measuring instruments used in the testing process (test equipment).

Software updates (see ISO 23783-1:2022, 6.9) are presumed to invalidate the calibration unless they are accompanied by a manufacturer's declaration that the volumetric performance of the system is not affected by the software update.

6.1.4 As-found and as-left reporting

When an instrument for calibration has been adjusted or repaired, the calibration results before and after adjustment or repair, if available, shall be reported.

6.1.5 Calibration interval recommendation

A calibration certificate (or calibration label) should contain a recommendation on the calibration interval, based upon the requirements of the intended use of the ALHS, if these are known.

Annex A
(informative)

Applications of descriptive statistics

A.1 General

This annex includes four examples showing how the descriptive statistics can be applied to the testing of automated liquid handling systems (ALHS). Each example includes a description of the test design including plate layouts, an arrangement of 'measurement results' in plate layout format, and calculations for various descriptive statistics using the formulae given in Clause 5.

Example "measurement results" are not representative of the performance of any particular real ALHS. Instead, these results were generated using a random number generator. Nevertheless, they may provide useful examples of how the descriptive statistics can be applied to data arranged in plate format and used to evaluate the volumetric performance of any ALHS configuration.

A short discussion of test design can be found in A.2.

A.2 Test design

A.2.1 General

The examples in this annex illustrate the flexibility inherent in the indexing systems described in Clause 5. Every test design should include a decision about the elements shown in Table A.1. The first three elements [channels (L), replicates (N), and experiments (M)] relate to the l,m,n indexing scheme described in Clause 5.3. For measurements made in microplates, it is necessary to define the plate density (e.g. micro analytical 96- or 384-wells) along with the number of plates that will be used for the testing.

Table A.1 — Experimental design for each example

Element	Symbol	Example 1 (A.3)	Example 2 (A.4)	Example 3 (A.5)	Example 4 (A.6)
ALHS channels	L	8	8	96	8
Reproducible experiments	M	1	3	1	1
Replicates/experiment	N	12	12	3	48
Wells	W	96	96	96	384
Number of microplates	P	1	3	3	1
Individual measurements	–	96	288	288	384

A.2.2 Plate layouts

In addition to the information in Table A.1, it is important to define the position and order that each channel delivers liquid into the microplate or set of microplates. Figures A.1 to A.3 show the order that is used in the examples found in this annex.

Figure A.1 shows a common layout when delivering into a 96-well plate using an eight-channel ALHS. In Figure A.1, the eight channels proceed left to right, so that the first delivery from each channel is into the first column, then repeats in order across the entire plate, for a total of 12 deliveries per channel. This plate layout is used in Examples 1 and 2.

		Column Number											
Channel	Row	1	2	3	4	5	6	7	8	9	10	11	12
$l = 1$	A	$n = 1$	$n = 2$	$n = 3$	$n = 4$	$n = 5$	$n = 6$	$n = 7$	$n = 8$	$n = 9$	$n = 10$	$n = 11$	$n = 12$
$l = 2$	B	$n = 1$	$n = 2$	$n = 3$	$n = 4$	$n = 5$	$n = 6$	$n = 7$	$n = 8$	$n = 9$	$n = 10$	$n = 11$	$n = 12$
$l = 3$	C	$n = 1$	$n = 2$	$n = 3$	$n = 4$	$n = 5$	$n = 6$	$n = 7$	$n = 8$	$n = 9$	$n = 10$	$n = 11$	$n = 12$
$l = 4$	D	$n = 1$	$n = 2$	$n = 3$	$n = 4$	$n = 5$	$n = 6$	$n = 7$	$n = 8$	$n = 9$	$n = 10$	$n = 11$	$n = 12$
$l = 5$	E	$n = 1$	$n = 2$	$n = 3$	$n = 4$	$n = 5$	$n = 6$	$n = 7$	$n = 8$	$n = 9$	$n = 10$	$n = 11$	$n = 12$
$l = 6$	F	$n = 1$	$n = 2$	$n = 3$	$n = 4$	$n = 5$	$n = 6$	$n = 7$	$n = 8$	$n = 9$	$n = 10$	$n = 11$	$n = 12$
$l = 7$	G	$n = 1$	$n = 2$	$n = 3$	$n = 4$	$n = 5$	$n = 6$	$n = 7$	$n = 8$	$n = 9$	$n = 10$	$n = 11$	$n = 12$
$l = 8$	H	$n = 1$	$n = 2$	$n = 3$	$n = 4$	$n = 5$	$n = 6$	$n = 7$	$n = 8$	$n = 9$	$n = 10$	$n = 11$	$n = 12$

Figure A.1 — Plate layout for eight channels dispensing into a 96-well plate

Figure A.2 shows a seemingly trivial example where a 96-channel head is used to deliver into a 96-well plate. Each well in the plate contains replicate number 1 as shown in Figure A.2 a). To collect additional replicates per channel, it is necessary to use additional plates. Figure A.2 b) shows the pattern for numbering channels when it is necessary to label channels by number rather than using a row and column address. This plate layout is used in Example 3.

	Column Number											
Row	1	2	3	4	5	6	7	8	9	10	11	12
A	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$
B	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$
C	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$
D	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$
E	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$
F	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$
G	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$
H	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 1$

a) Replicate number for one 96-channel dispense

	Column Number											
Row	1	2	3	4	5	6	7	8	9	10	11	12
A	$l = 1$	$l = 2$	$l = 3$	$l = 4$	$l = 5$	$l = 6$	$l = 7$	$l = 8$	$l = 9$	$l = 10$	$l = 11$	$l = 12$
B	$l = 13$	$l = 14$	$l = 15$	$l = 16$	$l = 17$	$l = 18$	$l = 19$	$l = 20$	$l = 21$	$l = 22$	$l = 23$	$l = 24$
C	$l = 25$	$l = 26$	$l = 27$	$l = 28$	$l = 29$	$l = 30$	$l = 31$	$l = 32$	$l = 33$	$l = 34$	$l = 35$	$l = 36$
D	$l = 37$	$l = 38$	$l = 39$	$l = 40$	$l = 41$	$l = 42$	$l = 43$	$l = 44$	$l = 45$	$l = 46$	$l = 47$	$l = 48$
E	$l = 49$	$l = 50$	$l = 51$	$l = 52$	$l = 53$	$l = 54$	$l = 55$	$l = 56$	$l = 57$	$l = 58$	$l = 59$	$l = 60$
F	$l = 61$	$l = 62$	$l = 63$	$l = 64$	$l = 65$	$l = 66$	$l = 67$	$l = 68$	$l = 69$	$l = 70$	$l = 71$	$l = 72$
G	$l = 73$	$l = 74$	$l = 75$	$l = 76$	$l = 77$	$l = 78$	$l = 79$	$l = 80$	$l = 81$	$l = 82$	$l = 83$	$l = 84$
H	$l = 85$	$l = 86$	$l = 87$	$l = 88$	$l = 89$	$l = 90$	$l = 91$	$l = 92$	$l = 93$	$l = 94$	$l = 95$	$l = 96$

b) Channel numbers of the 96-channel head

Figure A.2 — Plate layout for 96-channels dispensing into a 96-well plate

Figure A.3 shows a layout where an eight-channel head delivers into a 384-well plate. In this layout, the eight channels make the first delivery (n = 1) into the first column of alternating rows (i.e. wells A1, C1, E1, ..., O1). Then the channels proceed left to right across the entire plate until reaching the end of the row (column 24, in this example). Next, each channel moves downward and continues delivering while travelling right to left along the second row to complete the operation. This plate layout is used in Example 4.

		Column Number											
Channel	Row	1	2	3	4	5	6	7	8	9	10	11	12
l = 1	A	n = 1	n = 2	n = 3	n = 4	n = 5	n = 6	n = 7	n = 8	n = 9	n = 10	n = 11	n = 12
	B	n = 48	n = 47	n = 46	n = 45	n = 44	n = 43	n = 42	n = 41	n = 40	n = 39	n = 38	n = 37
l = 2	C	n = 1	n = 2	n = 3	n = 4	n = 5	n = 6	n = 7	n = 8	n = 9	n = 10	n = 11	n = 12
	D	n = 48	n = 47	n = 46	n = 45	n = 44	n = 43	n = 42	n = 41	n = 40	n = 39	n = 38	n = 37
l = 3	E	n = 1	n = 2	n = 3	n = 4	n = 5	n = 6	n = 7	n = 8	n = 9	n = 10	n = 11	n = 12
	F	n = 48	n = 47	n = 46	n = 45	n = 44	n = 43	n = 42	n = 41	n = 40	n = 39	n = 38	n = 37
l = 4	G	n = 1	n = 2	n = 3	n = 4	n = 5	n = 6	n = 7	n = 8	n = 9	n = 10	n = 11	n = 12
	H	n = 48	n = 47	n = 46	n = 45	n = 44	n = 43	n = 42	n = 41	n = 40	n = 39	n = 38	n = 37
l = 5	I	n = 1	n = 2	n = 3	n = 4	n = 5	n = 6	n = 7	n = 8	n = 9	n = 10	n = 11	n = 12
	J	n = 48	n = 47	n = 46	n = 45	n = 44	n = 43	n = 42	n = 41	n = 40	n = 39	n = 38	n = 37
l = 6	K	n = 1	n = 2	n = 3	n = 4	n = 5	n = 6	n = 7	n = 8	n = 9	n = 10	n = 11	n = 12
	L	n = 48	n = 47	n = 46	n = 45	n = 44	n = 43	n = 42	n = 41	n = 40	n = 39	n = 38	n = 37
l = 7	M	n = 1	n = 2	n = 3	n = 4	n = 5	n = 6	n = 7	n = 8	n = 9	n = 10	n = 11	n = 12
	N	n = 48	n = 47	n = 46	n = 45	n = 44	n = 43	n = 42	n = 41	n = 40	n = 39	n = 38	n = 37
l = 8	O	n = 1	n = 2	n = 3	n = 4	n = 5	n = 6	n = 7	n = 8	n = 9	n = 10	n = 11	n = 12
	P	n = 48	n = 47	n = 46	n = 45	n = 44	n = 43	n = 42	n = 41	n = 40	n = 39	n = 38	n = 37

Column Number											
13	14	15	16	17	18	19	20	21	22	23	24
n = 13	n = 14	n = 15	n = 16	n = 17	n = 18	n = 19	n = 20	n = 21	n = 22	n = 23	n = 24
n = 36	n = 35	n = 34	n = 33	n = 32	n = 31	n = 30	n = 29	n = 28	n = 27	n = 26	n = 25
n = 13	n = 14	n = 15	n = 16	n = 17	n = 18	n = 19	n = 20	n = 21	n = 22	n = 23	n = 24
n = 36	n = 35	n = 34	n = 33	n = 32	n = 31	n = 30	n = 29	n = 28	n = 27	n = 26	n = 25
n = 13	n = 14	n = 15	n = 16	n = 17	n = 18	n = 19	n = 20	n = 21	n = 22	n = 23	n = 24
n = 36	n = 35	n = 34	n = 33	n = 32	n = 31	n = 30	n = 29	n = 28	n = 27	n = 26	n = 25
n = 13	n = 14	n = 15	n = 16	n = 17	n = 18	n = 19	n = 20	n = 21	n = 22	n = 23	n = 24
n = 36	n = 35	n = 34	n = 33	n = 32	n = 31	n = 30	n = 29	n = 28	n = 27	n = 26	n = 25
n = 13	n = 14	n = 15	n = 16	n = 17	n = 18	n = 19	n = 20	n = 21	n = 22	n = 23	n = 24
n = 36	n = 35	n = 34	n = 33	n = 32	n = 31	n = 30	n = 29	n = 28	n = 27	n = 26	n = 25
n = 13	n = 14	n = 15	n = 16	n = 17	n = 18	n = 19	n = 20	n = 21	n = 22	n = 23	n = 24
n = 36	n = 35	n = 34	n = 33	n = 32	n = 31	n = 30	n = 29	n = 28	n = 27	n = 26	n = 25
n = 13	n = 14	n = 15	n = 16	n = 17	n = 18	n = 19	n = 20	n = 21	n = 22	n = 23	n = 24
n = 36	n = 35	n = 34	n = 33	n = 32	n = 31	n = 30	n = 29	n = 28	n = 27	n = 26	n = 25
n = 13	n = 14	n = 15	n = 16	n = 17	n = 18	n = 19	n = 20	n = 21	n = 22	n = 23	n = 24
n = 36	n = 35	n = 34	n = 33	n = 32	n = 31	n = 30	n = 29	n = 28	n = 27	n = 26	n = 25

Figure A.3 — Plate layout for 8 channels dispensing into a 384-well plate

A.3 Example 1 — Eight channels dispensed into a single 96-well plate

A.3.1 Test design and measurement results

The test designed for this example is an eight-channel ALHS delivering 12 replicates into a 96-well plate. The plate layout is as shown in Figure A.1, and the test is accomplished in a single experiment using only one plate. The measurement results and calculated descriptive statistics, including reference to the formulae used, are shown in Figure A.4.

Example 1
8 channel device, L = 8
one single experiment, M = 1
12 replicates per channel, N = 12
measured in one 96-well plate

Channel		1	2	3	4	5	6	7	8	9	10	11	12
l = 1	A	97,87	97,72	98,33	98,99	98,79	97,98	98,43	99,32	95,69	99,46	99,23	97,59
l = 2	B	97,60	99,53	98,27	99,98	98,44	100,42	100,28	98,54	97,37	100,84	99,95	102,07
l = 3	C	98,23	96,62	95,95	96,14	98,24	96,79	97,77	97,93	98,31	96,28	96,99	97,53
l = 4	D	102,49	99,09	100,74	100,41	101,02	100,15	100,12	100,53	102,11	99,94	99,19	102,36
l = 5	E	97,31	96,41	98,07	97,97	96,73	99,27	99,13	98,58	96,93	98,30	97,35	98,31
l = 6	F	96,53	96,35	97,54	95,95	95,55	97,79	97,99	97,48	98,87	98,62	97,42	97,65
l = 7	G	97,11	97,13	99,12	97,16	96,53	97,10	98,15	97,14	99,45	97,33	98,16	98,05
l = 8	H	97,86	95,60	98,50	99,43	98,56	98,37	97,11	98,53	97,35	97,47	97,78	97,57

Channel Statistics

Form. (1)	Form. (3)	Form. (7)
$\overline{V}(l)$	$\eta_S(l)$	$C_V(l)$
98,28	-1,72 %	1,06 %
99,44	-0,56 %	1,42 %
97,23	-2,77 %	0,90 %
100,68	0,68 %	1,13 %
97,86	-2,14 %	0,94 %
97,31	-2,69 %	1,05 %
97,70	-2,30 %	0,91 %
97,84	-2,16 %	0,99 %
max. $\eta_S(l)$	0,68 %	
min. $\eta_S(l)$	-2,77 %	
max. $C_V(l)$	1,42 %	

Run Order Statistics

	n	1	2	3	4	5	6	7	8	9	10	11	12
Form. (9)	$\overline{V}(n)$	98,13	97,30	98,32	98,25	97,98	98,48	98,62	98,50	98,26	98,53	98,26	98,89
Form. (3)	$\eta_S(n)$	-1,87 %	-2,70 %	-1,68 %	-1,75 %	-2,02 %	-1,52 %	-1,38 %	-1,50 %	-1,74 %	-1,47 %	-1,74 %	-1,11 %
Form. (11)	$C_V(n)$	1,88 %	1,42 %	1,38 %	1,75 %	1,73 %	1,36 %	1,14 %	1,09 %	1,99 %	1,53 %	1,09 %	2,09 %

Overall Statistics	
Grand Avg	Form. (12)
$\overline{V}_{GA}$	98,29

Overall CV	Form. (13)
$C_{V,OA}$	1,53 %

Form. (14)
$C_{V,C2C}$
1,21 %

Figure A.4 — Results and calculated statistics for Example 1

A.3.2 Statistics results

Channel statistics are shown to the right of the data. The eight channel averages are calculated by applying Formula (1) to each of the eight rows, and relative systematic error (η_S) is calculated by comparing each of these channel averages to the target volume using Formula (3). Channel CV, $C_V(l)$, is calculated for each row using Formula (7).

Run order statistics are shown below the data. The 12 run order mean volumes, $\overline{V}(n)$, are calculated using Formula (9) and the relative systematic error is calculated by using Formula (3). The run order CV, $C_V(n)$, is calculated by applying Formula (11) to the eight measurements in each column.

Overall statistics are shown in the bottom left part of Figure A.4. The grand average volume ($\overline{V}_{GA}$) and overall CV ($C_{V,OA}$) are calculated using Formulae (12) and (13), respectively.

The channel-to-channel CV ($C_{V,C2C}$) is shown in the lower right and is calculated using the eight channel mean volumes ($\overline{V}(l)$) in the column shown above the channel-to-channel CV box in Figure A.4. In this example, the overall CV result is 1,53 %, which is somewhat larger than the channel-to-channel CV (1,21 %). Also, the maximum channel CV is 1,42 % (maximum of all the CVs calculated by Formula (7). This example shows that both channel-to-channel differences and channel CV contribute to overall CV.

A.4 Example 2 — Eight channels dispensed into three 96-well plates

A.4.1 Test design and measurement results

The test design for this example is an extension of Example 1, again with an eight-channel ALHS delivering 12 replicates into a 96-well plate. The plate layout is as shown in Figure A.1. However, in Example 2 the test consists of three separate experiments using three plates (one plate per experiment). The measurement results and calculated descriptive statistics are shown in Figure A.5. This example shows how multiple reproducible experiments may be combined for more thorough testing.

A.4.2 Statistics results for Example 2

Channel statistics are shown to the right of each set of plate data. The eight channel averages are calculated by applying Formula (1) to each of the eight rows, and relative systematic error is calculated by comparing each of these channel averages to the target volume using Formula (3). Channel CV is calculated for each row using Formula (7).

NOTE Because the test was defined to be three different experiments, three different sets of channel statistics are calculated here. However, if the test had been defined as one experiment across three plates (one run with $N = 36$) then only one set of channel statistics would have been calculated. It is for this reason important to precisely define the test in order to know how the data is to be analysed.

Run order statistics are shown below the last plate of data. The 12 run order mean volumes are calculated using Formula (9) which includes a summation over the $M = 3$ experiments; 24 data points are averaged for each of these calculated means. The relative systematic error is calculated by using Formula (3). The run order CV is calculated by applying Formula (11) to the eight measurements in each column for each plate (24 volume measurement points for each run order CV result).

Overall statistics are shown in the bottom left part of Figure A.5. The grand average volume and overall CV are calculated using Formulae (12) and (13), respectively.

Because this test includes three experiments, it is possible to calculate channel statistics which are combined from all three experiments. These are shown in the lower right and use Formulae (4) and (8).

Lastly, the channel-to-channel CV is shown in the extreme lower right in Figure A.5 and is calculated using the eight channel mean volumes calculated by Formula (4) and shown in the column above the channel-to-channel CV box. When multiple experiments are included in a single test, Formula (14) shows that the combined channel statistics should be used, as is illustrated in this example.

The overall CV result in Example 2 is similar to the channel-to-channel CV. From this, it can be seen that channel-to-channel differences are the dominant contributor to overall CV in this example.

