INTERNATIONAL WORKSHOP AGREEMENT

IWA 15

First edition
2015-10-15

Specification and method for the determination of performance of automated liquid handling systems

Spécification et méthode pour la détermination de performance des systèmes automatisés de manipulation de liquides

Reference number
IWA 15:2015(E)

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

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 on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TMBG, *Technical Management Board Groups*.

Specification and method for the determination of performance of automated liquid handling systems

1 Scope

This International Workshop Agreement (IWA) specifies methods for testing the volumetric performance of air-displacement, system-liquid filled and positive displacement automated liquid handling systems (ALHS), including an estimation of measurement uncertainties and established traceability to reference standards (preferably, traceability to SI Units). The testing methods specified in this document may also be used to measure the volumetric performance of automated liquid delivery systems which do not aspirate the test liquid.

This IWA also specifies statistical methods for the determination of random and systematic errors (including intra-plate and inter-plate comparisons), analysis of measured results when using multichannel dispensing heads, and analysis depending on dispense patterns. It further defines terms and formulae to be used for summarizing test results.

This IWA also specifies the information to be provided to users of ALHS, including the display of summary results and performance claims.

This IWA 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 microplates. Manipulation of the microplates on the deck of the automated liquid handling system may be achieved automatically, semi-automatically, or manually.

This IWA addresses the needs of:

— suppliers of ALHS, as a basis for quality control including, where appropriate, the issuance of supplier's declarations;

— test houses and other bodies, as a basis for independent certification and calibration;

— users of the equipment, to enable calibration, verification, validation, and routine testing of trueness and precision.

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

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

3 Terms and definitions

For purposes of this document, the following terms and definitions apply.

3.1 Definitions

3.1.1
accuracy
<automated liquid handling system> closeness of agreement between a delivered volume and the target volume

Note 1 to entry: The concept 'accuracy' is not a quantity and is not given a numerical quantity value. A liquid delivery is said to be more accurate when it is accomplished with a smaller liquid handling error.

[SOURCE: ISO/IEC Guide 99:2007, 2.13, modified]

3.1.2
air displacement
liquid handling principle in which a body of air is contained between the piston and the test liquid

Note 1 to entry: It is possible to have a large air gap (piston systems), or smaller air gap between the test liquid and the system liquid (liquid filled systems).

3.1.3
ALHS uncertainty
non-negative parameter characterizing the dispersion of the measured volumes relative to the target volume

Note 1 to entry: Uncertainty is inversely related to accuracy, and is a quantity value. This value should be expressed in accordance with ISO/IEC Guide 98-3.

[SOURCE: ISO/IEC Guide 99:2007, 2.26, modified]

3.1.4
aliquot
<automated liquid handling system> single delivery during a multi-dispense

3.1.5
automated liquid handling system
ALHS
system with a complete, installed liquid handling device, including tips and other essential parts needed for delivering a specified volume without human intervention into microplates

Note 1 to entry: Examples of automated liquid handling systems include automated pipetting systems (APS), and automated dispensing systems (ADS).

3.1.6
calibration
<automated liquid handling system> operation that, under specified conditions, establishes a relation between the target volume of the ALHS and the delivered volume

Note 1 to entry: A calibration may be expressed by a statement, a calibration curve or a calibration table. It may include a correction, but correction or adjustment is not a required element of a calibration.

[SOURCE: ISO/IEC Guide 99:2007, 2.39, modified]

3.1.7
dead air volume
captive air volume
air gap
<piston-operated automated liquid handling systems> air volume between the lower part of the piston and the surface of the aspirated liquid

Note 1 to entry: It is possible to have a large air gap (piston systems), or smaller air gap for liquid filled systems. Sometimes called captive air volume.

Note 2 to entry: Commonly, an air gap can be adjusted through ALHS system parameters, while the dead air volume or captive air volume cannot be adjusted (see 4.2.1).

3.1.8
delivered volume
quantity delivered by a liquid handling system

Note 1 to entry: Delivered volume is a conceptual term and cannot be known with complete certainty due to measurement error.

3.1.9
dispense height
height at which the test liquid is dispensed relative to a stated reference

3.1.10
dispensing system
system for delivering liquids from a pre-filled liquid reservoir

3.1.11
disposable tip
tip, which is attached once and after use, as defined by the manufacturer, detached and intended to be discarded

Note 1 to entry: Disposable tips are usually made of plastic.

Note 2 to entry: Disposable tips are in contrast to fixed tips, which are described in 4.3.4.

3.1.12
dry contact dispensing
dispensing of liquid while tip is in contact with the dry target

3.1.13
factory acceptance testing
internal vendor testing to ensure ALHS performance to specifications

3.1.14
forward mode pipetting
direct mode pipetting
pipetting mode where the entire aspirated volume is delivered

3.1.15
immersion depth
depth of the tip below the liquid surface

Note 1 to entry: Immersion depth can be applied to both aspiration and dispensing (wet contact).

[SOURCE: Toolpoint Photometric Volume Check Procedure:2008, modified]

3.1.16
individually controlled channel
liquid handling channel that can be operated independently of other channels

3.1.17
labware
<automated liquid handling systems> materials used in conjunction with liquid handling operations

Note 1 to entry: Labware includes disposable tips, reservoirs, receiving vessels, adapters and microplates.

3.1.18
maximum permissible error
upper or lower permitted extreme value for the deviation of the dispensed volume from the target volume

3.1.19
maximum specified volume
largest volume for which the manufacturer offers specifications

Note 1 to entry: The maximum specified volume may vary depending on instrument configuration (e.g. disposable tip size, syringe size).

3.1.20
measured volume
quantity reported by a volume measuring system

Note 1 to entry: In practice, all measurements contain some measurement error. The measured volume is a quantity value and serves as an estimate of the delivered volume which is not known with complete certainty.

3.1.21
measurement method
measurement procedure
detailed description of a measurement according to one or more measurement principles

Note 1 to entry: The source document (ISO/IEC Guide 99) draws a distinction between 'measurement method' and 'measurement procedure' and that distinction is ignored here. In this IWA, the terms are used interchangeably.

Note 2 to entry: The measurement method descriptions in this IWA detail the steps needed to make a volume measurement and calculate certain descriptive statistics. Additional details needed to operate the ALHS are part of the 'test process' as defined in 3.1.43. In this IWA, the measurement method is one of the components of a 'test process.'

[SOURCE: ISO/IEC Guide 99:2007, 2.6, modified]

3.1.22
measurement uncertainty
<measured volume> non-negative parameter characterizing the dispersion of the measured volumes relative to the delivered volume

Note 1 to entry: Uncertainty is inversely related to accuracy, and is a quantity value. This value should be expressed in accordance with the ISO/IEC Guide 98-3.

[SOURCE: ISO/IEC Guide 99:2007, 2.26, modified]

3.1.23
metrological traceability
property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty

Note 1 to entry: Additional information can be found in the notes to definition (ISO/IEC Guide 99:2007, 2.41) and the related term 'metrological traceability chain' (ISO/IEC Guide 99:2007, 2.42).

[SOURCE: ISO/IEC Guide 99:2007, 2.41]

3.1.24
microplate
flat plate with an array of wells

Note 1 to entry: Some dimensions of microplates are defined in ANSI/SLAS standards.[1-5]

3.1.25
minimum specified volume
smallest volume for which the manufacturer offers specifications

Note 1 to entry: The minimum specified volume may vary depending on instrument configuration.

3.1.26
multichannel head
group of liquid handling channels operated in common

Note 1 to entry: Common arrangements of multichannel heads include 8, 96, 384, and 1536 channel heads. Other arrangements are possible, e.g. 2 channel to 1536 channel configurations.

Note 2 to entry: Pipetting channels in a multichannel head may be controlled by a single, common drive, or each channel may be controlled individually.

3.1.27
multi dispense
repeat dispense
sequential dispense
<automated pipetting systems> a collection of dispenses without intervening aspiration

Note 1 to entry: First dispense can be different, and is frequently wasted.

Note 2 to entry: Repeat dispenses usually dispense repeatedly the same volume, while sequential dispenses usually dispense different volumes.

3.1.28
non-contact dispensing
contact-free dispensing
free-jet dispensing
dispensing of the liquid while tip is in air and without contacting the target or the liquid contained in the target

3.1.29
outlier
member of a set of values which is inconsistent with the other members of that set

3.1.30
pipetting system
system for aspirating and dispensing a specified volume of liquid

[SOURCE: Toolpoint Gravimetric Volume Check Procedure:2008, modified]

3.1.31
positive displacement
direct displacement
liquid handling principle in which a mechanical actuator is in direct contact with the test liquid

3.1.32
precision
<of liquid handling system> the closeness of agreement between the measured volume of independent delivered volumes under stipulated conditions

Note 1 to entry: Precision is conceptual and not a quantity value.

Note 2 to entry: Measurement precision is usually expressed numerically by measures of random error, such as standard deviation, variance, or coefficient of variation under the specified conditions of measurement.

Note 3 to entry: The 'stipulated conditions' can be, for example, repeatability conditions of measurement, intermediate precision conditions of measurement, or reproducibility conditions of measurement (see ISO 5725-1:1994).

[SOURCE: ISO 5725-1:1994, 3.12, modified]

3.1.33
random error
<automated liquid handling systems> component of liquid handling error that in replicate deliveries varies in an unpredictable manner

[SOURCE: ISO/IEC Guide 99:2007, 2.19, modified]

3.1.34
reservoir
liquid container
vessel that contains the liquid

3.1.35
reverse mode pipetting
pipetting mode in which excess volume is aspirated and remains in the tip after delivery

3.1.36
single dispense
individual dispense
<automated pipetting systems> single dispense per aspiration

3.1.37
site acceptance testing
in-situ testing at the user's site, typically part of the installation process

3.1.38
supplier's declaration
document by which a supplier gives written assurance that an ALHS conforms to the requirements of one or more commonly accepted industry standards

Note 1 to entry: This IWA can be referenced as an applicable industry standard.

3.1.39
systematic error
<automated liquid handling system> component of volumetric error that in replicate deliveries remains constant or varies in a predictable manner

Note 1 to entry: Systematic error is estimated by calculating the average volume of a series of deliveries and comparing it to the indicated volume of the automated liquid handling system. Frequently this result is expressed as a percentage of the indicated volume.

[SOURCE: ISO/IEC Guide 99:2007, 2.17, modified]

3.1.40
system liquid
liquid used to transmit energy between a mechanical actuator and the test liquid

Note 1 to entry: System liquids can reduce or completely eliminate system dead air volume.

Note 2 to entry: System liquid is usually deionized water. For special applications organic solvents such as DMSO or aqueous solutions such as saline (e.g. 0,9 % NaCl) can be used.

Note 3 to entry: System liquid can be used for flushing and rinsing tips to minimize cross contamination.

3.1.41
target volume
indicated volume
selected volume
volume which is intended to be delivered

3.1.42
test liquid
liquid used for the volume measurement

Note 1 to entry: May be aqueous or other solvents. Aqueous test liquids can be pure water or contain other compounds such as buffers, dyes or salts. The chemical composition of the test liquid can vary significantly depending on method.

3.1.43
test process
<automated liquid handling system> detailed description of an ALHS testing procedure including system operation and measurement method

Note 1 to entry: The test process includes all details needed to reproduce the test or interpret the results. The measurement method is defined in 3.1.21, and is only a part of the test process.

3.1.44
test report
document reporting the result of the testing

Note 1 to entry: Details regarding information contained in test reports is specified in Clause 8.

3.1.45
test result
value of a characteristic obtained by carrying out a specified test method

Note 1 to entry: Test result is a broader concept than measured volume. The test result can be a single measured volume, a set of measured volumes, or descriptive statistics such as the mean or standard deviation of multiple measurements. The test method should specify what form the test results take.

[SOURCE: ISO 5725-1:1994, 3.2]

3.1.46
traceability
metrological traceability
property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty

[SOURCE: ISO/IEC Guide 99:2007, 2.41]

3.1.47
trueness
<automated liquid handling system> closeness of agreement between the average volume delivered in a large series of deliveries and the target volume

Note 1 to entry: Trueness is inversely related to systematic error, but is not related to random error.

[SOURCE: ISO/IEC Guide 99:2007, 2.14, modified]

3.1.48
validation
<automated liquid handling system> confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled

Note 1 to entry: The term “validated” is used to designate the corresponding status.

Note 2 to entry: The test protocol for this testing should reflect the liquid volumes and instrument settings, at which the ALHS will be operated.

Note 3 to entry: A product may meet all of its specifications (verification), but that does not ensure that it will work in the operating paradigm (validation).

3.1.49
verification
<automated liquid handling system> confirmation, through provision of objective evidence, that volumetric performance specifications have been fulfilled

Note 1 to entry: The term “verified” is used to designate the corresponding status.

Note 2 to entry: Volumetric performance specifications may vary depending on the environment where the ALHS is used, e.g. factory and field specifications may be different.

[SOURCE: ISO/IEC Guide 99:2007, 2.44, modified]

3.1.50
wet contact dispensing
dispensing of the test liquid while tip is in contact with liquid present in the target

3.2 Abbreviated terms

See Table 1.

Table 1 — Abbreviated terms used in this document

Abbreviated term	Explanation
ADS	Automated Dispensing System
ALHS	Automated Liquid Handling System
APS	Automated Pipetting System
C2C	Channel-to-Channel
CV	Coefficient of Variation
DI	De-ionized
DMSO	Dimethylsulfoxide
GA	Grand Average
HVAC	Heating, Ventilation, and Air Conditioning
IEC	International Electrotechnical Commission
IWA	International Workshop Agreement
MU	Measurement Uncertainty
MW	Molecular weight
NaOH	Sodium hydroxide
OA	Over All
OD	Optical Density
p.a.	*pro analysi* (purity grade of chemicals)
p-NP	*para*-nitrophenol
RH	Relative Humidity [%]
RSE	Relative Systematic Error
rcf	Relative centrifugal force
rpm	Revolutions per minute
SD	Standard Deviation
SMOW	Surface Mean Ocean Water

4 Operation of automated liquid handling systems

4.1 Types of automated liquid handling systems

The purpose of this clause is to provide examples of some types of ALHS. This list is not intended to be a comprehensive list as technologies continue to evolve.[6]

4.1.1 Types of piston operated automated liquid handling systems

Automated liquid handling systems can operate as follows:

— variable volume; designed by the manufacturer to aspirate and dispense volumes selectable by the user within the specified useful volume range of the dispense head and selected tips, for example between 10 µl and 100 µl.

— a larger volume may be aspirated into the tips, followed by a series of subsequent dispenses of smaller aliquots.

The piston can

— either have a body of air contained between the piston and the surface of the liquid (air-displacement), or

— be in direct contact with the surface of the liquid (positive or direct displacement), or

— be in contact with a system liquid.

The system can

— have a single tip, or

— have multiple tips, operated by individual pistons, or

— have multiple tips, operated by a single, common drive or moving plate with multiple pistons simultaneously driven by a common drive.

The tip can be

— permanently attached to the dispense channel of the ALHS, or

— disposable, and used for one or more aspirate and dispense sequences.

4.1.2 Types of other (pump operated) automated liquid handling systems

Pump operated automated liquid handling systems can operate as follows:

variable volume; designed by the manufacturer to dispense volumes selectable by the user within the specified useful volume range of the dispense head.

The dispense head can

— be permanently attached to the instrument, or

— be exchangeable, e.g. to change the usable volume range or number of channels.

The system can

— have a peristaltic or diaphragm pump to aspirate liquid from a reservoir, or

— have a pressurized liquid reservoir and separate liquid valves to control the liquid delivery.