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Contents

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

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Introduction

The examples given in this document are informative and support the requirement found in the ISO 23783 series to perform an estimation of measurement uncertainty when calibrating automated liquid handling systems (ALHS) according to the measurement procedures described in ISO 23783-2. The examples in this document are based on the principles of ISO/IEC Guide 98-3.

Automated liquid handling systems – Uncertainty of the measurement procedures

1 Scope

This document describes the measurement uncertainty analysis of the measurement procedures described in ISO 23783-2, following the approach described in ISO/IEC Guide 98-3.

This document also includes the determination of other uncertainty components related to the liquid delivery process and the device under test (DUT) to estimate the overall measurement uncertainty of delivered volumes by an automated liquid handling system (ALHS).

2 Normative references

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

ISO/IEC Guide 98-3, *Uncertainty of measurement — Part 3: Guide to the expression of uncertainty in measurement (GUM:1995)*

ISO/IEC Guide 99, *International vocabulary of metrology — Basic and general concepts and associated terms (VIM)*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 23783-1, ISO/IEC Guide 98-3, and ISO/IEC Guide 99 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 General procedure for the uncertainty calculation

The evaluation of measurement uncertainty in this document follows the ISO/IEC Guide 98-3 “Guide to the Expression of Uncertainty in Measurement (GUM).” The method has the following steps:

a) Expressing, in mathematical terms, the relationship between the measurand and its input quantities.

b) Determining the expected value of each input quantity.

c) Determining the standard uncertainty of each input quantity.

d) Determining the degree of freedom for each input quantity.

e) Determining all covariance between the input quantities.

f) Calculating the expected value for the measurand.

g) Calculating the sensitivity coefficient of each input quantity.

h Calculating the combined standard uncertainty of the measurand.

i) Calculating the effective degrees of freedom of the combined standard uncertainty.

j) Choosing an appropriate coverage factor, *k*, to achieve the required confidence level.

k) Calculating the expanded uncertainty.

In this document, the uncertainty of the measurement procedure is separated in three different clauses:

- the uncertainty components associated with the measuring system, see Clause 6;
- the uncertainty components associated with the device under test (ALHS), see Clause 7;
- the uncertainty components associated with the liquid delivery process, see Clause 8.

5 Modelling of the measurement

Each measurement procedure has specific uncertainty components associated with the measuring system. These uncertainty components are described in the respective annex for each procedure. See Annex A for the dual-dye ratiometric photometric procedure, Annex B for the gravimetric procedure, and Annex C for the optical image analysis of droplets.

6 Standard uncertainty components associated with the measuring system

6.1 General information on standard uncertainty components estimation

It is possible to experimentally estimate the standard uncertainty of measurement, $u(x)$, for a quantity x, by performing multiple measurements of x under repeatability conditions. This is called a type A evaluation according to ISO/IEC Guide 98-3. The standard deviation of the obtained values is a measure of the repeatability of the measurement. The standard uncertainty associated with x can be a standard deviation based on previous experience (in the case where a single measurement of x is made), or the standard deviation of the mean equal to stdev(x)/sqrt(n) (in the case where x is the average of n readings).

See ISO Guide 98-3:2008, 4.2 for more information on type A evaluation of standard uncertainty.

In addition to repeated measurements, the systematic component of the uncertainty of measurement for a quantity x is estimated by other means. This is called a type B evaluation according to ISO/IEC Guide 98-3. For example, one can obtain information for that estimation by considering the manufacturer's specifications of the ALHS (e.g., resolution, linearity, drift, temperature dependence).

Often the manufacturer's specifications are given in the form of an interval covering the measurement value, with no additional information regarding distribution or coverage. In those cases, the measurement can be assumed to follow a uniform or rectangular distribution. This distribution is characterised by a constant probability inside the interval while the probability outside the interval is zero.

The interval can be used in a type B evaluation to give the variance of x in the form shown in Formula (1):

$$u^2(x) = \frac{(a_+ - a_-)^2}{12} \tag{1}$$

where

$u^2(x)$ is the variance of the variable x;

a_+ and a_- give the upper and lower limits of the interval of the variable x.

The standard uncertainty, $u(x)$, is given as the square root of the variance.

In addition to uniform rectangular, other distributions are also possible when performing type B evaluations. See ISO/IEC Guide 98-3:2008, 4.3 for more information on type B evaluations of standard uncertainty.

6.2 Specific information on standard uncertainty components estimation

Specific information regarding standard uncertainty components particular to the dual-dye ratiometric photometric, gravimetric, and optical volume measurement procedures is given in Annex A, Annex B and Annex C, respectively.

7 Standard uncertainty components associated with the ALHS

7.1 General

Subclauses 7.2 and 7.3 describe uncertainty components, which can influence the operation and performance of the ALHS. Depending on the specific type of ALHS and liquid used, additional uncertainty components can be identified.

7.2 ALHS-type specific influencing parameters

The following parameters can impact the liquid delivery process, depending on the type of ALHS used:

- exchangeable components, e.g., type of tips and others (see ISO 23783-1:2022, 6.8);
- air cushion effects (if applicable, e.g., for air-displacement or liquid-filled piston-operated ALHS with an air gap);
- system liquid effects:
  - dissolved gases in the system liquid,
  - internal dilution effect of the sample with system liquid,
  - temperature sensitivity of the system liquid;
- environmental effects on the deck of the ALHS:
  - rate of air flow,
  - air temperature,
  - relative humidity,
  - barometric pressure;
- vibration effects;
- electrostatic effects.

7.3 Test liquid properties influencing ALHS operation

- surface tension;
- viscosity;
- density;
- content of dissolved gas in the test liquid (formation of micro-bubbles);
- vapor pressure;
- Weber number (contact-free dispensing), see Reference [1].

7.4 Standard uncertainty of ALHS resolution

The standard uncertainty related to the resolution can be determined according to Formula (2):

$$u(res)=\frac{\Delta res}{\sqrt{12}} \tag{2}$$

where

$u(res)$ is the standard uncertainty related to the resolution of the ALHS volume selection device;

Δres is the actual or estimated resolution of the volume selection device of the ALHS.

NOTE 1 The uncertainty related to the resolution of the ALHS is included in the uncertainty budget when the measurements are dependent on the direct reading of the output volume. The uncertainty of the resolution is also included when estimating the uncertainty of the systematic error e_S.

NOTE 2 The physical resolution of an ALHS is not necessarily the resolution displayed in the ALHS' software. For example, different syringe sizes can be mounted on the same stepper motor. The resolution of the stepper motor will translate into various volume resolutions, depending on the size of the syringe mounted on the motor.

7.5 Standard uncertainty of cubic expansion coefficient (optional)

The correction of a measured volume at test temperature to a reference temperature is optional, see ISO 23783-2:2022, Clause 7. If this correction is performed, this subclause applies.

The standard uncertainty related to the cubic expansion coefficient γ is dependent on knowledge of the actual material of the artefact and on the source of the data, which provides an appropriate value. Data from the literature or manufacturer can be used for the expansion coefficient, and this value would be expected to have a relative standard uncertainty of 5 % to 10 % of the expansion coefficient, see Reference [2].

For devices with an air cushion, the thermal effects on the cubic expansion coefficient and the air cushion are entangled and need to be considered in tandem or determined experimentally. The details of this entanglement are beyond the scope of this document.

7.6 Standard uncertainty associated with air cushion effects (optional)

If applicable, the standard uncertainty related to the air cushion effect $u(\Delta V_{\text{cush}})$ depends on the size of the air cushion that is related to the lifting height in the pipette tip and can be calculated according to Formula (3), which is based on the information given in DKD-R 8-1:2011, 8.7, see Reference [3]:

$$u(\Delta V_{\text{cush}})=\sqrt{\left(u(V\Delta p)\times c_{V\Delta p}\right)^2+\left(u(V\Delta h_r)\times c_{V\Delta h_r}\right)^2+\left(u(V\Delta t_{\text{L}})\times c_{V\Delta t_{\text{L}}}\right)^2} \tag{3}$$

where

$u(\Delta V_{\text{cush}})$ is the standard uncertainty related to the air cushion effect;

$u(V\Delta p)$ is the standard uncertainty attributed to air pressure variation during the tests;

$u(V\Delta h_r)$ is the standard uncertainty attributed to the humidity variation during the tests;

$u(V\Delta t_{\text{L}})$ is the standard uncertainty caused by variation between the test liquid temperature, air temperature and temperature of the ALHS under calibration;

c_i are the sensitivity coefficients related to each uncertainty component.

The variations of each parameter are determined experimentally during the test, and only apply to ALHS which have an air cushion. Variations of these parameters are influenced by the size of the air cushion relative to the volume of the aspirated test liquid, the test liquid's vapor pressure, and whether the tip has been pre-wetted or not.

The sensitivity coefficients c_i related to the air cushion effect from pressure, relative humidity and temperature can be derived from DKD-R 8-1, see Reference [3].

8 Repeatability and reproducibility of the liquid delivery process

8.1 Repeatability (experimental standard deviation)

Annexes A, B and C allow the determination of the standard uncertainties associated with the respective measurement procedure. To derive the standard uncertainty associated with the liquid delivery process, the experimental standard deviation needs to be included. When the mean delivered volume is the measurand, the standard deviation s_r is divided by the square root of the number of repeated measurements n, as shown in Formula (4):

$$s_r(\overline{V}) = \frac{s_r}{\sqrt{n}} \tag{4}$$

where

$s_r(\overline{V})$	is the standard deviation of the mean volume $\overline{V}$;
s_r	is the repeatability standard deviation;
n	is the number replicate measurements.

8.2 Reproducibility

The uncertainty related to the reproducibility of $\overline{V}$ (from one test of the ALHS to the next test) also needs to be included. There are several methods to determine this uncertainty contribution:

a) A laboratory can perform experimental studies where the ALHS test is performed multiple times under different reproducibility conditions (see also ISO 23783-1:2022, 3.40 "reproducibility" and ISO 23783-3:2022, 5.3.2 "experiment") and the reproducibility standard deviation of the measurement result $\overline{V}$ is calculated, symbol $s_d(\overline{V})$;

b) If no such information is available, a value for reproducibility of the selected volume can be provided by ALHS manufacturers or third parties. As no further information on the variation of individual measurements is taken into account, a rectangular distribution is suggested.

NOTE Sometimes, the value for reproducibility can be inferred from the declared "accuracy" or "systematic error" of the ALHS. For example, an ALHS with a 5 % specification for accuracy (systematic error) can be used to estimate the reproducibility as 2,9 % according to Formula (1).

The influence of environmental conditions on the reproducibility of ALHS performance can vary depending on the type of ALHS used and needs to be determined experimentally.

9 Combined standard uncertainty of measurement associated with the systematic error of mean volume

According to ISO/IEC Guide 98-3, when the errors of input quantities are uncorrelated, the variance characterising the uncertainty of measurement can be written according to Formula (5):

$$u^2 = \sum_i c_i^2 \times u^2(x_i) \tag{5}$$

where:

u^2 is the variance characterizing the uncertainty of measurement;

$u^2(x_i)$ are the variances associated with each input quantity which contributes to the final result (described by the model);

c_i^2 are the squares of the sensitivity coefficients giving the degree of influence of each individual standard uncertainty.

The sensitivity coefficients can be determined by evaluating the partial derivatives of the measurement equation, by numerical simulations, or by physical experiment. In the case of this technical report, it is possible to obtain explicit functions for many sensitivity coefficients by evaluating the partial derivatives as shown in Clause 10.

In this document, the uncertainty components are described in groups corresponding to Clauses 6, 7 and 8. For the mean volume of a calibration or test, Formula (6) applies.

$$u^2(\bar{V}) = u_{\mathrm{MS}}^2(\bar{V}) + u_{\mathrm{ALHS}}^2(\bar{V}) + u_{\mathrm{LDP}}^2(\bar{V}) \tag{6}$$

where

$u^2(\bar{V})$ is the variance characterising the uncertainty of the mean volume in a test or calibration;

$u_{\mathrm{MS}}^2(\bar{V})$ is the variance characterising the uncertainty due to the measuring system;

$u_{\mathrm{ALHS}}^2(\bar{V})$ is the variance characterising the uncertainty due to the ALHS;

$u_{\mathrm{LDP}}^2(\bar{V})$ is the variance characterising the uncertainty due to the liquid delivery process.

When reporting uncertainty of the mean delivered volume (n replicates), the two liquid delivery process variances (see Clause 8) are combined as shown in Formula (7).

$$u_{\mathrm{LDP}}^2(\bar{V}) = \frac{s_r^2}{n} + s_d^2(\bar{V}) \tag{7}$$

where

s_r is the repeatability standard deviation;

$\frac{s_r^2}{n}$ is the variance of the mean volume due to the repeatability of the ALHS; and

$s_d^2(\bar{V})$ is the variance of the mean volume due to test process reproducibility.

10 Sensitivity coefficients

The sensitivity coefficients for the measurement procedure can be derived using any of the following approaches:

a) from the mathematical model of the measurement;

b) experimentally from comparison studies;

c) derived and reported as relative values as percent of the measurand (e.g., EURAMET cg-18 for air density, Reference [4]);

d) available from literature values;

e) numerical simulation.

Sensitivity coefficients specific to each type of measurement procedure (photometric, gravimetric and optical) are given in Annex A, Annex B and Annex C, respectively.

11 Coverage factor *k*

In order to calculate an appropriate coverage factor *k* for a 95 % confidence level (see ISO/IEC Guide 98-3:2008, Annex G), the effective degrees of freedom ν_{eff} are estimated by means of the Welch-Satterthwaite equation as shown in Formula (8):

$$\nu_{\mathrm{eff}} = \frac{u_V^4}{\sum_{i=1}^{n} \frac{u_i^4}{\nu_i}} \tag{8}$$

where

ν_{eff} are the effective degrees of freedom for the measurement;

u_V is the combined standard uncertainty of the measured volume;

u_i is the standard uncertainty of each component;

ν_i are the degrees of freedom of each component.

For 10 or more measurements, *k* can be calculated or *k* = 2 can be used if the individual standard uncertainty values have a similar weight in the combined uncertainty. For less than 10 measurements, *k* is calculated.

12 Expanded uncertainty of measurement associated with the mean volume

The expanded uncertainty of the mean volume $\bar{V}$ is expressed according to Formula (9), where the standard uncertainty is multiplied by the coverage factor *k*.

$$U(\bar{V}) = u(\bar{V}) \times k \tag{9}$$

where

$U(\bar{V})$ is the expanded uncertainty of the mean volume;

$u(\bar{V})$ is the standard uncertainty of the mean volume;

k is the coverage factor.

13 Examples for determining the uncertainty of the volume measurement of ALHS

13.1 Measurement conditions

When reporting measurement results and the associated uncertainty information, it is necessary to understand and interpret the report. A comprehensive list of required and recommended information to be included in the reports is found in ISO 23783-3:2022, Clause 6. The uncertainty related to the reproducibility of $\bar{V}$ (see 8.2) also needs to be included.

For each example in this report, measurement conditions are described in appropriate sub-clauses within the Annexes.

13.2 Results

13.2.1 Standard uncertainty of the ALHS mean volume

The standard uncertainty of the mean delivered volume is calculated according to Formula (10):

$$u(\bar{V})=\sqrt{u_{\mathrm{MS}}^{2}(\bar{V})+u_{\mathrm{ALHS}}^{2}(\bar{V})+u_{\mathrm{LDP}}^{2}(\bar{V})} \qquad (10)$$

13.2.2 Expanded uncertainty of the measurement

The expanded uncertainty of the measurement is calculated by multiplying the standard uncertainty of the measurement by the coverage factor *k,* according to Formula (11). The numerical value of *k* is reported as part of the result.

$$U(\bar{V})=u(\bar{V})\times k \qquad (k=x_k) \qquad (11)$$

where x_k is the value of *k* used in the calculation of the expanded uncertainty of the mean volume.

NOTE 1 This expanded uncertainty is the measurement uncertainty of the calibration as described in ISO 23783-3:2022, 6.1.3 a).

13.2.3 Result of measurement

The overall result of the measurement, including the expanded uncertainty of measurement, can be expressed as shown in Formula (12).

$$V_{\mathrm{M}}=\bar{V}\pm U(\bar{V}) \qquad (k=x_k) \qquad (12)$$

$$V_{\mathrm{M}} = 5{,}014\ \mu\mathrm{l} \pm 0{,}012\ \mu\mathrm{l}\ (k = 2)$$

where V_{M} is the overall result of the measurement including the expanded uncertainty.

NOTE The numerical values are given for illustration only. The particular values for each example are given in each Annex.

13.2.4 Caution regarding use of numerical values in this report

The numerical values of the quantity estimation, standard uncertainty, and sensitivity coefficients are applicable to the specific situation described in each example and are often volume dependent. It is not appropriate to use the values given in this technical report for other situations or volumes.

13.2.5 Remarks on conformity with ISO/IEC Guide 98-3

The term “random error” as used in the ISO 23783 series is equivalent to the term “experimental standard deviation” used in ISO/IEC Guide 98-3.

There is no direct equivalent to “systematic error” e_{S} as used in the ISO 23783 series that is found within ISO/IEC Guide 98-3.

NOTE 1 The term “instrumental bias” is found in ISO/IEC Guide 99 and is similar to “systematic error of measurement” provided the ALHS is considered a liquid measuring instrument and care is taken regarding a positive or negative numerical sign in the result.

To evaluate the uncertainty of the systematic error of measurement, a volume difference V_{D} can be defined, as shown in Formula (13).

$$V_{\mathrm{D}}=V_{\mathrm{S}}-\bar{V} \qquad (13)$$