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Guide for volume determination within the scope of reference measurement procedures in medical reference measurement laboratories
Part 1: Calibration liquid water

Foreword

This Guide was drawn up by the working group "Measuring Devices" of the Technical Committee "Medical Quantities" of the Deutscher Kalibrierdienst (DKD, German Calibration Service) and the Advisory Council for Metrology in Medicine at the Physikalisch-Technische Bundesanstalt (PTB). After a testing period the Guide will be revised, if necessary, and probably be published as a DKD Guidance Publication.

1 Purpose

1.1 Fields of application

The Guide is intended for operators of calibration laboratories accredited for measurands relevant to medicine. It establishes metrological requirements for the calibration of measuring devices used to determine the volume of a liquid within the scope of reference measurement procedures in medical reference measurement laboratories - also referred to as medical target value laboratories.

Part 1 of the Guide describes the gravimetric calibration of measuring devices used to determine the volume of a liquid, using water as the calibration liquid. In part 2, the gravimetric calibration with liquids other than water (for example: protein containing solutions, sera, organic solutions) will be described.

The Guide is to serve to determine and represent also the expanded uncertainty of a volume dispensing process using suitable procedures. It is applicable to the following measuring devices used to determine the volume of a liquid (in brackets standards are stated which specify the respective calibration procedures):

-	Volumetric flasks	(DIN EN ISO 1042, DIN 12664-1, DIN 12664-2)
-	Volumetric pipettes	(DIN 12687, DIN 12688, DIN 12691, DIN 12690)
-	Measuring pipettes	(DIN 12689, DIN 12695, DIN 12696, DIN 12 697, DIN 12 699)
-	Piston pipettes	(DIN EN ISO 8655-2, DIN EN ISO 8655-6)
-	Piston burettes	(DIN EN ISO 8655-3, DIN EN ISO 8655-6)
-	Dilutors	(DIN EN ISO 8655-4, DIN EN ISO 8655-6)
-	Dispensers	(DIN EN ISO 8655-5, DIN EN ISO 8655-6)
-	Microlitre syringes	

2 Measuring instruments and their traceability to national standards

2.1 Weighing

The weighing instrument is the most important measuring instrument for the gravimetric calibration of measuring devices used to determine the volume of liquid. For the operation of this device, the instructions of the "Guide for the determination of the mass within the scope of reference measurement procedures in medical reference measurement laboratories" [1] are to be noted. Traceability to the national standard of mass in particular is to be carried out in accordance with the "Guide for the determination of the mass within the scope of reference measurement procedures in medical reference measurement laboratories."

2.1.1 Selection of a suitable weighing instrument

The uncertainty of measurement assigned to the measurement of a liquid volume can be split up into two uncertainty contributions:

- a contribution u_D to be assigned to the measurement of the volume with a volumetric apparatus (i.e. dispensing of the volume with a dispensing device)

and

- a contribution u_G to be assigned to the gravimetric determination of the liquid volume already dispensed with the weighing instrument.

From these contributions, the uncertainty of measurement is calculated as follows:

$$u_V = \sqrt{u_D^2 + u_G^2} \qquad (1)$$

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An important objective of the calibration is the determination of the contribution u_D, i.e. of the measurement uncertainty of the dispensing process of the dispensing device to be calibrated. Determination of u_D from the experimental standard deviation determined by the calibration is easy if the contribution u_G is clearly smaller than u_D (standard value: smaller than one third). In this case, $u_V = u_D$ can be set.

The standard uncertainty u_W assigned to the measurement with the weighing instrument generally furnishes the greatest uncertainty contribution to u_G. To meet the requirement $u_G < u_D/3$ it must, therefore, be ensured by selection of a suitable weighing instrument that u_W is as small as possible.

Table 1 shows minimum requirements for actual scale intervals d and uncertainties of measurement u_w of electronic weighing instruments to allow the above requirement $u_G << u_D$ to be met.

Table 1: Examples of required actual scale intervals of electronic weighing instruments

Volume V of the weighed-in quantity	Required resolution d of the weighing instrument	Maximum uncertainty of measurement u_w
1 µl to 10 µl	0,001 mg	0,002 mg
above 10 µl to 100 µl	0,01 mg	0,02 mg
above 100 µl to 1000 µl	0,1 mg	0,2 mg
above 1 ml to 10 ml	0,1 mg	0,2 mg
above 10 ml to 200 ml	1 mg	2 mg
above 200 ml to 1000 ml	10 mg	20 mg

2.2 Other measuring instruments

In addition, the measuring instruments stated in Table 2 should be available:

Table 2: Other measuring instruments

Instrument	Measuring range	Resolution	Recommended period for recalibration
Thermometers [1]	10 °C to 30 °C	0,1 K	mercury-in-glass thermometers: 10 years electrical resistance thermometers: 5 years thermistors/thermocouples: 1 year
Barometers [2]	950 hPa to 1050 hPa	1 hPa	2 years
Chronometers [3]	15 min	1 s	
Hygrometers [4]	20 % to 80 %	5%	

[1]   for the measurement of ambient and water temperature, required to determine the density of water and air
[2]   for the measurement of the air pressure, required to determine the density of air
[3]   for the measurement of discharge and waiting times
[4]   for the control of the relative air humidity in the laboratory room

Thermometers and barometers must have been calibrated or verified against standards which are traceable to the national standards. If the difference between the calibration results of two successive calibrations (long-term drift) for a measuring instrument exceeds the uncertainty of measurement, it should be checked whether the device can be replaced by another one of better stability or if the conditions of use can be improved.

2.3 Calibration liquid

Water of quality 2 according to DIN ISO 3696 [2] serves as calibration liquid.

3 Ambient conditions

As regards the ambient conditions, the values stated in Table 3 should be complied with.

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Table 3: Ambient conditions to be met

Ambient conditions	Tolerance
Air temperature	15 °C to 25 °C
Maximum change of the air temperature per hour without new adjustment of the weighing instrument	± 1 K
Relative humidity of the air	35 % to 65 %

The influence of ambient temperature changes on the weighing process can be prevented by readjustment of the weighing instrument after each temperature change.

Air flows, shocks and unilateral thermal radiation as well as dusts which might exert an influence on the weighing process should be avoided in the measurement rooms. Construction-physical measures for thermal insulation should be taken to prevent heat transmission by solar radiation through the windows. Where appropriate, air-conditioning should be provided.

4   Preparation of the gravimetric calibration of volumetric apparatus

4.1   Specification of the volumes to be calibrated

The volumes are to be calibrated for which the volume meter is to be used.

4.2   Error sources during calibration

Frequent error sources:

-       insufficient cleanness of the measuring instruments (due, for example, to unsuitable laboratory detergents)

-       wetting of measuring instrument components which are not part of the measuring space

-       air bubbles on the internal wall of the measuring space

-       air bubbles in the discharge facility

-       extension of discharge time

-       non-observance of waiting time

-       inaccurate adjustment of the meniscus

Other possible error sources as stated in general technical rules are also to be observed. Cf. in particular PTB Testing Instructions, volume 21, Volume meters for Laboratory Use [3].

To prevent insufficient cleanness of measuring instruments from leading to non-uniform wetting and irregular discharge of measuring instruments set to "Ex", the measuring instruments should be cleaned again shortly before gravimetric calibration is carried out. This is not applicable to plastic pipette tips of the disposable type which are normally used without additional cleaning. For cleaning, it is usually sufficient to use a commercial laboratory detergent or an ultrasonic bath filled with detergent. After cleaning, the measuring instruments must repeatedly be thoroughly rinsed with water of quality 2 or 3 according to DIN ISO 3696 [2] and dried.

4.3   Meniscus adjustment

For measuring instruments which are used to determine the volume of liquids and which, for calibration, are filled up to a mark, the liquid meniscus must be adjusted so that its lowest point and the upper edge of the mark (ring mark, scale mark) lie in one plane when observed free from parallax.

The adjustment is to be carried out with the measuring instrument in a perpendicular position. It is to be ensured that the volume meters are not wetted in addition to the wetting above the mark which is required for the exact formation and adjustment of the meniscus.

4.4   Temperature regulation of measuring instruments and calibration liquid

Measuring instruments, volumetric apparatus and calibration liquid are to be stored in the measurement room for at least 1 hour before the measurements are started to adjust their temperature to the temperature of the measurement room. The reservoir for the calibration liquid must be closed to avoid evaporative cooling.

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4.5 Evaporation influence

An important uncertainty contribution to the gravimetric volume determination of water stems from the evaporation of the liquid. Evaporation leads to a decrease in the water mass during weighing. In addition, the temperature of liquid and measuring instrument and the relative humidity of the air may be influenced by evaporation. Suitable measures must be taken (evaporation trap in the weighing instrument, closed reservoir, closed weighing vessel, short measuring duration) to minimize the evaporation influence. In the case of very small volumes (smaller than 10 µl), the evaporation must be additionally compensated by calculation.

5 Carrying-out of the calibration

5.1 Procedure

Calibration is carried out by weighing the water volume filled into the measuring instrument (adjustment to "In") or delivered by the measuring instrument by discharge or displacement with a piston (adjustment to "Ex").

During calibration, the temperatures of calibration liquid and measuring devices shall not deviate by more than 1 K from the temperature of the calibration room.

If the volumetric apparatus is adjusted to "In", the empty measuring instrument is first tared on a suitable weighing instrument. The weighed value W_1 is obtained from the indication of the weighing instrument. The measuring instrument is then filled with calibration liquid up to the mark of the volume to be calibrated. After that, the filled measuring instrument is weighed. From the indication of the weighing instrument the weighed value W_2 is obtained.

If the volumetric apparatus is adjusted to "Ex", a weighing vessel is first tared on a suitable weighing instrument. The weighed value W_1 is obtained from the indication of the weighing instrument. The calibration liquid is then discharged from the volumetric apparatus into the weighing vessel. Then the weighing vessel is weighed again. From the indication of the weighing instrument the weighed value W_2 is obtained.

The weighed value W of the calibration liquid which was contained in or discharged from the instrument, is the difference $W_2 - W_1$. The weighed value W is the uncorrected difference between the indications of the weighing instrument.

Both weighings are to be carried out, if possible, in a short interval of time to guarantee identical conditions and to keep evaporation losses as small as possible. The weighing vessel should be touched only at the edge to avoid heating or residues of hand sweat. If necessary, gloves are to be used. When smaller volumes are weighed, it is recommended to use gripping devices (e.g. tweezers) when handling the weighing vessel and to cover the vessel. The weighing vessel used should go with the weighing instrument, the dispensed volume and a prefilling possibly made.

Note:

A prefilling in the weighing vessel leads to more uniform evaporation effects and thus reduces measurement deviations caused by evaporation during calibration.

The measurement values are to be stated in the calibration records as follows:

- weighed values W_1 and W_2 correct to the resolution d of the weighing instrument;
- temperature t_{FL} of the calibration liquid correct to 0,1 K;
- ambient temperature t_L correct to 0,1 K;
- air pressure p correct to 1 hPa;
- relative air humidity φ correct to 10 %.

The volume must be determined at least ten times under identical conditions.

5.2 Evaluation of the calibration

The volume V is calculated according to the following equation:

$$V = W \cdot Z \cdot Y \tag{2}$$

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In this equation, W is the net weighed value ($W_2 - W_1$), Z comprises the air buoyancy correction of the weighed value and the conversion of the mass of the water measured into volume at measurement temperature, and Y is a thermal expansion correction for the volume of the measuring instrument to be applied if measurement and reference temperature are different.

$$Z = \frac{1}{\rho_W} \cdot \frac{1 - \dfrac{\rho_L}{\rho_G}}{1 - \dfrac{\rho_L}{\rho_W}} = \frac{1}{\rho_G} \cdot \frac{\rho_G - \rho_L}{\rho_W - \rho_L} \tag{3}$$

$$Y = 1 - \alpha_c \cdot (t_d - t_b) \tag{4}$$

The following thus applies:

$$V = (W_2 - W_1)\left(\frac{1}{\rho_W - \rho_L}\right)\left(1 - \frac{\rho_L}{\rho_G}\right)(1 - \alpha_c(t_d - t_b)) \tag{5}$$

where:

V	volume in ml
W_2	weighed value of the vessel with calibration liquid in g
W_1	weighed value of the empty vessel in g
ρ_L	density of the air in g/cm³
ρ_G	density of the weights in g/cm³ (standardized value for steel weights: 8000 kg/m³ = 8 g/cm³)
ρ_W	density of the calibration liquid in g/cm³
α_c	cubic expansion coefficient in 1/K
t_d	temperature of the volumetric apparatus in °C (for simplification equated with the temperature of the calibration liquid)
t_b	reference temperature in °C

The water density ρ_W can be taken from Table A (Annex 1) and the air density ρ_L from Table B (Annex 2) or calculated with the formulas stated.

A thermal expansion correction can be applied if the volume is to be calculated at a temperature other than the measurement temperature. In the case of glass instruments, the cubic expansion coefficient α_c can normally be used for borosilicate glass. Values for expansion coefficients are given in Table 4.

As different materials also have different expansion coefficients, this expansion correction cannot, however, be applied to multicomponent systems (in particular piston pipettes). In single-component systems, as a result of stress in the material, the expansion coefficient is often not homogeneous for the whole volumetric apparatus either. It is, therefore, recommended to perform the calibrations at the proposed temperature of use and to avoid the thermal expansion correction.

Table 4: Cubic thermal expansion coefficient α_c for conventional device materials

Material	Cubic expansion coefficient α_c
borosilicate glass (Duran, Pyrex, Rasotherm)	$9{,}9 \cdot 10^{-6}$ 1/K
semi-borosilicate glass (e.g. Durobax, Fiolax, device glass 20):	$14{,}7 \cdot 10^{-6}$ 1/K
general soft soda glass (lime-soda glass, e. g. AR glass):	$27{,}0 \cdot 10^{-6}$ 1/K
plastics	$300 \cdot 10^{-6}$ 1/K to $600 \cdot 10^{-6}$ 1/K

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5.3 Calculation of the standard measurement uncertainty and of the mean value

The mean value, the experimental standard deviation of the single values and the experimental standard deviation of the mean value can be directly determined from the gravimetric volume measurement carried out n times.

The mean value $\overline{V}$ is obtained from:

$$\overline{V} = \frac{1}{n} \sum_{i=1}^{n} V_i \tag{6}$$

The experimental standard deviation of the single values results from:

$$s(V_i) = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} \left(V_i - \overline{V} \right)^2} \tag{7}$$

The experimental standard deviation of the mean value is:

$$s(\overline{V}) = \frac{s(V_i)}{\sqrt{n}} \tag{8}$$

If n is large enough (recommended: $n \geq 10$), $s(\overline{V})$ describes the standard measurement uncertainty u_v of the measurement of volume V.

This experimental standard deviation contains uncertainty components of both, the dispensing process and the gravimetric volume determination. If measuring instruments meeting the requirements stated in section 2 are used, the uncertainty component to be assigned to gravimetric volume determination is usually considerably smaller than the component to be assigned to the volume measurement by the volume meter (dispensing device). The measured experimental standard deviation can, therefore, be considered to be attributable solely to the dispensing device. The uncertainty of measurement can then be assigned to the volume measurement by the dispensing device, without splitting the uncertainties (cf. section 2.1). If there are doubts, for example in the case of very small volumes of $V < 10\,\mu l$, a measurement uncertainty budget must be established for gravimetric volume determination, to be able to decide whether the requirement that u_G must be clearly smaller than u_D (standard value: smaller than one third) is met. This uncertainty of measurement is calculated in accordance with "Guide to the Expression of Uncertainty in Measurement" [4] (cf. also [5] and [6]). It is combined of the components from all measuring instruments involved (weighing instrument, thermometer, barometer). Its calculation requires knowledge of the measurement uncertainties of all measuring instruments involved in the measuring process and of their sensitivity coefficients describing the weighting of the respective measurement uncertainties for the measurement uncertainty assigned to the volume measured. An example is given in Annex 3.

5.4 Statement of the calibration results

The result must state the expanded uncertainty of measurement obtained by multiplying the standard uncertainty by the coverage factor $k = 2$.

$$U = k \cdot u_v \tag{9}$$

In the final statement, the **numerical value of the uncertainty of measurement** is to be stated with maximally two significant digits. The value must be rounded up whenever rounding down would change the uncertainty by more than 5 %. The **numerical value of the measurement result** is to be rounded to the last significant digit of the expanded uncertainty of measurement. The usual rules valid for the rounding of numbers (see ISO 31-0, Annex B [7]) also apply to the rounding of the measurement result. For some contributions to the uncertainty of measurement it may, however, be suitable to retain more than two significant digits to avoid greater rounding deviations in the following calculations.

The calibration result is to be stated in one of the following forms:

Example: volume $V = 100,35\,\mu l$, averaged over 10 single measurements
                experimental standard deviation of the single values: $0,40\,\mu l$
                experimental standard deviation of the mean value: $0,40\,\mu l / \sqrt{10} = 0,126\,\mu l$
                expanded uncertainty of measurement of the mean value $U = 0,25\,\mu l$, $k = 2$
                relative expanded uncertainty of measurement of the mean value $U/V = 0,25$ %, $k = 2$

   a) $V = 100,35\,(1 \pm 0,0025)\,\mu l$

   b)   Complete measurement result for the volume: $100,35\,(1 \pm 0,0025)\,\mu l$

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   c) $V = 100{,}35\ \mu l \pm 0{,}25\ \mu l$

   d)   Complete measurement result for the volume: $100{,}35\ \mu l \pm 0{,}25\ \mu l$

   e) $V = 100{,}35\ \mu l$; $U/V = 0{,}0025$

   f) $V = 100{,}35\ \mu l$; $U/V = 0{,}25\ \%$

The statement of the measurement result is to be supplemented by the following explanation:

"The expanded uncertainty stated is the product of the standard uncertainty and the coverage factor $k = 2$. For a normal distribution it corresponds to a coverage probability of approx. 95%."

Note: If the assigned uncertainty is stated for a single dispensing process with the respective volumetric apparatus, the **experimental standard deviation of the single values** is to be used instead of the experimental standard deviation of the mean value!

6   Literature

[1]   *Leitfaden für die Massebestimmung bei Referenzmessprozeduren in medizinischen Referenzlaboratorien* (Guide for the determination of the mass within the scope of reference measurement procedures in medical reference measurement laboratories), PTB-Mitt. **109** (1999) No. 5, pp. 379-383

[2]   DIN ISO 3696 *Wasser für analytische Laborzwecke - Spezifikationen und Prüfverfahren* (Water for analytical laboratory use – specification and test procedures)

[3]   PTB-Prüfregeln Band 21: *Volumenmeßgeräte für Laboratoriumszwecke* (PTB Testing Instructions, volume 21: Volume meters for laboratory purposes), 1992, ISSN 0341-7964

[4]   *Guide to the Expression of Uncertainty in Measurement*, first edition 1993, corrected and reprinted 1995, International Organization for Standardization (Geneva, Switzerland)

[5]   EA-4/02 (EAL-R2): 1997: *Expression of the Uncertainty of Measurement in Calibration*

[6]   EA-4/02-S1 (EAL-R2-S1): 1997*: Supplement 1 to Expression of the Uncertainty of Measurement in Calibration - Examples*

[7]   ISO 31-0: 1992-08: *Quantities and Units; Part 0: General Principles*

[8]   F.E. Jones, G.L. Harris: ITS-90 *Density of Water Formulation for Volumetric Standards Calibration* Journal of Research of the National Institute of Standards and Technology 97 (1992) pp 335-340

[9]   G. S. Kell: *Density, Thermal Expansivity, and Compressibility of Liquid Water from 0 °C to 150 °C: Correlations and Tables for Atmospheric Pressure and Saturation Reviewed and Expressed on 1968 Temperature Scale* J. Chem. & Eng. Data **20** (1975) 97-105

[10]   H. Bettin und F. Spieweck: *Die Dichte des Wassers als Funktion der Temperatur nach Einführung der Internationalen Temperaturskala von 1990*, PTB-Mitteilungen **100**, (1990) No. 3, p. 195

[11]   F. Spieweck, H. Bettin: Review: *Solid and liquid density determination*, tm - Technisches Messen 59 (1992) pp. 237-244 and pp. 285-292

[12]   ISO Technical Report 20461: *Determination of uncertainty for volume measurements made using the gravimetric method*, first Edition, 2000

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Annex 1

Table A:

Water density as a function of the temperature according to the ITS-90 Kell formula for gas-free water. Water density in kg/m³, temperature t in °C

t	+0,0	+0,1	+0,2	+0,3	+0,4
15	999,099	999,084	999,069	999,053	999,038
16	998,942	998,926	998,910	998,893	998,876
17	998,774	998,757	998,739	998,722	998,704
18	998,595	998,576	998,558	998,539	998,520
19	998,404	998,385	998,365	998,345	998,325
20	998,203	998,182	998,162	998,141	998,120
21	997,991	997,970	997,948	997,926	997,904
22	997,769	997,747	997,724	997,701	997,678
23	997,537	997,513	997,490	997,446	997,442
24	997,295	997,270	997,246	997,221	997,196
25	997,043	997,018	996,992	996,966	996,940
26	996,782	996,755	996,729	996,702	996,675
27	996,511	996,484	996,456	996,428	996,401
28	996,232	996,203	996,174	996,146	996,117
29	995,943	995,913	995,884	995,854	995,825
30	995,645				

t	+0,5	+0,6	+0,7	+0,8	+0,9
15	999,022	999,006	998,991	998,975	998,959
16	998,860	998,843	998,826	998,809	998,792
17	998,686	998,668	998,650	998,632	998,613
18	998,501	998,482	998,463	998,443	998,424
19	998,305	998,285	998,265	998,244	998,244
20	998,099	998,077	998,056	998,035	998,013
21	997,882	997,859	997,837	997,815	997,792
22	997,654	997,631	997,608	997,584	997,561
23	997,417	997,393	997,369	997,344	997,320
24	997,170	997,145	997,120	997,094	997,069
25	996,914	996,888	996,861	996,835	996,809
26	996,648	996,621	996,594	996,566	996,539
27	996,373	996,345	996,316	996,288	996,260
28	996,088	996,059	996,030	996,001	995,972
29	995,795	995,765	995,735	995,705	995,675

The density of water ρ_w (in kg/m³) is given in a simple form by a formula (A1-1) by Jones and Harris [8] which, in the temperature range from 5 °C to 40 °C, is a very good approximation of the (more complex) formula by Kell [9]. In the temperature range from 5 °C to 40 °C, the relative deviation between this formula and the original formula by Kell (valid for temperatures between 0 °C and 150 °C, converted by Spieweck and Bettin in [10, 11] for the temperature scale ITS-90) is smaller than 10^{-6}.

$$\rho_w = \sum_{i=0}^{4} a_i t_w^i \qquad \text{(A1-1)}$$

with the constants (ITS-90 temperature scale)

$a_0 = 999{,}85308$ kg/m³

$a_1 = 6{,}32693 \cdot 10^{-2}$ °C^{-1} kg/m³

$a_2 = -8{,}523829 \cdot 10^{-3}$ °C^{-2} kg/m³

$a_3 = 6{,}943248 \cdot 10^{-5}$ °C^{-3} kg/m³

$a_4 = -3{,}821216 \cdot 10^{-7}$ °C^{-4} kg/m³

$t_w =$ water temperature in °C.

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Annex 2

Table B:

Air density for an air humidity of 50 % (CO_2 content of 0,04 %) as a function of air temperature and air pressure. Temperature scale: ITS-90, air density in kg/m³

T in °C	p_L in hPa											
	950	960	970	980	990	1000	1010	1020	1030	1040	1050	1060
15	1,145	1,157	1,169	1,181	1,193	1,206	1,218	1,230	1,242	1,254	1,266	1,278
16	1,141	1,153	1,165	1,177	1,189	1,201	1,213	1,225	1,237	1,249	1,261	1,273
17	1,137	1,149	1,161	1,173	1,185	1,197	1,209	1,221	1,233	1,245	1,257	1,269
18	1,132	1,144	1,156	1,168	1,180	1,192	1,204	1,216	1,228	1,240	1,252	1,264
19	1,128	1,140	1,152	1,164	1,176	1,188	1,200	1,212	1,224	1,236	1,248	1,259
20	1,124	1,136	1,148	1,160	1,172	1,183	1,195	1,207	1,219	1,231	1,243	1,255
21	1,120	1,132	1,144	1,155	1,167	1,179	1,191	1,203	1,215	1,227	1,238	1,250
22	1,116	1,128	1,139	1,151	1,163	1,175	1,187	1,198	1,210	1,222	1,234	1,246
23	1,112	1,123	1,135	1,147	1,159	1,170	1,182	1,194	1,206	1,217	1,229	1,241
24	1,107	1,119	1,131	1,143	1,154	1,166	1,178	1,190	1,201	1,213	1,225	1,236
25	1,103	1,115	1,127	1,138	1,150	1,162	1,173	1,185	1,197	1,209	1,220	1,232
26	1,099	1,111	1,122	1,134	1,146	1,157	1,169	1,181	1,192	1,204	1,216	1,227
27	1,095	1,107	1,118	1,130	1,142	1,153	1,165	1,176	1,188	1,200	1,211	1,223
28	1,091	1,103	1,114	1,126	1,137	1,149	1,160	1,172	1,184	1,195	1,207	1,218
29	1,087	1,098	1,110	1,121	1,133	1,145	1,156	1,168	1,179	1,191	1,202	1,214
30	1,083	1,094	1,106	1,117	1,129	1,140	1,152	1,163	1,175	1,186	1,198	1,209

The air density ρ_L (in kg/m³) is given by

$$\rho_L = \frac{k_1 p_L + \varphi \left(k_2 t_L + k_3 \right)}{t_L + t_{L0}} \tag{A2-1}$$

p_L is the air pressure in hPa, φ the relative air humidity in %, t_L the air temperature in °C.

The following coefficients are to be used:
$k_1 = 0,34844$ (kg/m³) · °C/hPa
$k_2 = -0,00252$ kg/m³
$k_3 = 0,020582$ (kg/m³) · °C
$t_{L0} = 273,15$ °C
See [10, 11].

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Annex 3

Example of a measurement uncertainty analysis according to the *"Guide to the Expression of Uncertainty in Measurement"* [4]

Tenfold gravimetric measurement of a nominal water volume of 100 µl, measured with a piston pipette. The measurements are carried out using a weighing instrument with a maximum useful load of 100 g and a resolution (actual scale interval) of 0,01 mg. The mean value from 10 single measurements is 100,35 µl, the experimental standard deviation $u_V = 0,40$ µl.

Model equation for the determination of the uncertainty u_G of the gravimetric determination of liquid volumes:

Preliminary remark:

The model equation has already been described in section 5.2 (basic equation), Annex 1 (water density) and Annex 2 (air density). In the following it will be shown in a somewhat modified form:

- A correction of the thermal expansion of the dispensing device has not been taken into account (Y in section 5.2 equation 4).

- All quantities are stated in SI base units. A scaling factor S has been introduced to allow the result to be stated in the practical unit of nanolitre.

- The weighing values (W_{01}, W_{02}) do not show any scattering and are assumed to be constants. Additional terms (w_{lin}, w_{evap}, w_{1read}, w_{1rep}, w_{2read}, w_{2rep}, w_{cal} and w_{drift}) to which an uncertainty of measurement has been assigned, describe the corrections necessary as a result of influences of weighing instrument and weighing process. The values of these terms are estimated to be 0 (zero) if they are additive and 1 (one) if they are multiplicative.

$$V = m/\rho_W \cdot S$$

$$m = W \cdot (1 - \rho_L/\rho_G)/(1 - \rho_L/\rho_W)$$

$$W = W_2 - W_1 + w_{lin} + w_{evap}$$

$$W_1 = (W_{01} + w_{1read} + w_{1rep}) \cdot w_{cal} \cdot w_{drift}$$

$$W_2 = (W_{02} + w_{2read} + w_{2rep}) \cdot w_{cal} \cdot w_{drift}$$

$$w_{drift} = 1 + t_{WD} \cdot t_{drift}$$

$$\rho_W = (a_0 + a_1 \cdot t_{Fl} + a_2 \cdot t_{Fl}^2 + a_3 \cdot t_{Fl}^3 + a_4 \cdot t_{Fl}^4) + \delta\rho_{approx}$$

$$\rho_L = (k_1 \cdot p_L - \varphi \cdot (k_2 \cdot t_L - k_3))/(t_L + t_{L0})$$

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List of quantities:

Quantity	Unit	Definition
W_{01}	kg	weighing value of empty measurement (tare)
W_{02}	kg	weighing value of full measurement (gross)
w_{1read}, w_{2read}	kg	correction for finite resolution (digital division) of weighing instrument
w_{1rep}, w_{2rep}	kg	reproducibility of weighing instrument
w_{lin}	kg	correction for deviations from linearity
w_{cal}		correction for calibration of weighing instrument
t_{WD}	K	temperature variation of weighing instrument
t_{drift}	K^{-1}	drift constant of weighing instrument
w_{evap}	kg	correction for evaporation
t_{Fl}	°C	liquid temperature (same value as t_W in Annex 1)
$\delta\rho_{approx}$	kg/m³	deviation of liquid density calculated with approximation formula (see Annex 1)
t_L	°C	air temperature
p_L	hPa	air pressure
φ	%	relative air humidity
ρ_G	kg/m³	density of steel weights (constant: 8000 kg/m³)
k_1	kg °C/hPa m³	constant k_1 for air density formula
k_2	kg/m³	constant k_2 for air density formula
k_3	kg °C/m³	constant k_3 for air density formula
t_{L0}	°C	constant t_{L0} for air density formula
a_0	kg/m³	constant a_0 for water density formula
a_1	kg/m³/°C	constant a_1 for water density formula
a_2	kg/m³/°C^2	constant a_2 for water density formula
a_3	kg/m³/°C^3	constant a_3 for water density formula
a_4	kg/m³/°C^4	constant a_4 for water density formula
S	nl/m³	scaling factor for conversion of m^3 into nl
Interim results		
m	kg	liquid mass
ρ_W	kg/m³	liquid density
ρ_L	kg/m³	air density
w_{drift}		deviation of weighed value due to temperature drift
W_1	kg	weighing value of empty measurement (tare) including corrections (without w_{lin} and w_{evap})
W_2	kg	weighing value of full measurement (gross) including corrections (without w_{lin} and w_{evap})
W	kg	weighing value of liquid including corrections, except for air buoyancy correction
Final result		
V	nl	measured volume of liquid

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W_{01}:	constant	value: 0 kg	
W_{02}:	constant	value: $100{,}065 \cdot 10^{-6}$ kg	
w_{1read}:	Type B rectangular distribution	value: 0 kg	half width of the limits: $5 \cdot 10^{-9}$ kg
w_{2read}:	Type B rectangular distribution	value: 0 kg	half width of the limits: $5 \cdot 10^{-9}$ kg
w_{1rep}:	Type B rectangular distribution	value: 0 kg	half width of the limits: $20 \cdot 10^{-9}$ kg
w_{2rep}:	Type B rectangular distribution	value: 0 kg	half width of the limits: $20 \cdot 10^{-9}$ kg
w_{lin}:	Type B rectangular distribution	value: 0 kg	half width of the limits: $20 \cdot 10^{-9}$ kg
w_{cal}:	Type B rectangular distribution	value: 1	half width of the limits: $1 \cdot 10^{-6}$
t_{WD}:	Type B rectangular distribution	value: 0 K	half width of the limits: 0,5 K
w_{evap}:	Type B rectangular distribution	value: 0 kg	half width of the limits: $0{,}5 \cdot 10^{-8}$ kg
t_{Fl}:	Type B rectangular distribution	value: 20 °C	half width of the limits: 0,1 K
$\delta\rho_{approx}$:	Type B rectangular distribution	value: 0 kg/m³	half width of the limits: 0,01 kg/m³
t_{L}:	Type B rectangular distribution	value: 20 °C	half width of the limits: 0,1 K
p_{L}:	Type B rectangular distribution	value: 1013 hPa	half width of the limits: 2 hPa
φ:	Type B rectangular distribution	value: 70 %	half width of the limits: 20 %
ρ_{G}:	constant	value: 8000 kg/m³	
k_{1}:	constant	value: 0,34844 kg °C/hPa m³	
k_{2}:	constant	value: 0,00252 kg/m³	
k_{3}:	constant	value: 0,020582 kg °C/m³	
T_{L0}:	constant	value: 273,15 °C	
a_{0}:	constant	value: 999,85308 kg/m³	
a_{1}:	constant	value: $6{,}32693 \cdot 10^{-2}$ kg/m³/°C	
a_{2}:	constant	value: $-8{,}523829 \cdot 10^{-3}$ kg/m³/°C^2	
a_{3}:	constant	value: $6{,}943248 \cdot 10^{-5}$ kg/m³/°C^3	
a_{4}:	constant	value: $-3{,}821216 \cdot 10^{-7}$ kg/m³/°C^4	
t_{drift}:	constant	value: $1 \cdot 10^{-6}$ °C^{-1}	
S:	constant	value: $1 \cdot 10^{12}$ nl/m³	
m:	interim result		
ρ_{W}:	interim result		
ρ_{L}:	interim result		
w_{drift}:	interim result		
W_{1}:	interim result		
W_{2}:	interim result		
W:	interim result		
V:	result		

Correlation: The input quantities are considered to be uncorrelated.

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Uncertainty budget:

Quantity	Estimate	Standard measurement uncertainty	Effective degrees of freedom	Sensitivity coefficient	Uncertainty contribution
W_{01}	0,0 kg	-	-	-	-
W_{02}	$100,065 \cdot 10^{-6}$ kg	-	-	-	-
w_{1read}	0,0 kg	$2,89 \cdot 10^{-9}$ kg	∞	$-1,0 \cdot 10^{9}$	$-2,9$ nl
w_{2read}	0,0 kg	$2,89 \cdot 10^{-9}$ kg	∞	$1,0 \cdot 10^{9}$	2,9 nl
w_{1rep}	0,0 kg	$11,5 \cdot 10^{-9}$ kg	∞	$-1,0 \cdot 10^{9}$	-12 nl
w_{2rep}	0,0 kg	$11,5 \cdot 10^{-9}$ kg	∞	$1,0 \cdot 10^{9}$	12 nl
w_{lin}	0,0 kg	$11,5 \cdot 10^{-9}$ kg	∞	$1,0 \cdot 10^{9}$	12 nl
w_{cal}	1	$577 \cdot 10^{-9}$	∞	$100 \cdot 10^{3}$	$58 \cdot 10^{-3}$ nl
t_{WD}	0,0 °C	$289 \cdot 10^{-3}$ °C	∞	$100 \cdot 10^{-3}$	$29 \cdot 10^{-3}$ nl
w_{evap}	0,0 kg	$2,89 \cdot 10^{-9}$ kg	∞	$1,0 \cdot 10^{9}$	2,9 nl
t_{Fl}	20,0 °C	$57,7 \cdot 10^{-3}$ °C	∞	18	1,1 nl
$\delta\rho_{approx}$	0,0 kg/m³	$2,89 \cdot 10^{-3}$ kg/m³	∞	-100	$-290 \cdot 10^{-3}$ nl
t_{L}	20,0 °C	$57,7 \cdot 10^{-3}$ °C	∞	$-410 \cdot 10^{-3}$	$-24 \cdot 10^{-3}$ nl
p_{L}	1013 hPa	1,15 hPa	∞	$100 \cdot 10^{-3}$	$120 \cdot 10^{-3}$ nl
φ	70 %	11,5 %	∞	$-9,0 \cdot 10^{-3}$	$-100 \cdot 10^{-3}$ nl
S	$1,0 \cdot 10^{-12}$ nl/m³	-	-	-	-
ρ_{G}	8000 kg/m³	-	-	-	-
t_{drift}	$1,0 \cdot 10^{-6}$ °C^{-1}	-	-	-	-
a_0	999,85308 kg/m³	-	-	-	-
a_1	0,0632693 kg/m³/°C	-	-	-	-
a_2	$-8,523829 \cdot 10^{-3}$ kg/m³/°C²	-	-	-	-
a_3	$6,943248 \cdot 10^{-5}$ kg/m³/°C³	-	-	-	-
k_1	0,34844 kg °C/hPa m³	-	-	-	-
k_2	0,00252 kg/m³	-	-	-	-
k_3	0,020582 kg °C/m³	-	-	-	-
t_{L0}	273,15 °C	-	-	-	-
V	$100,350 \cdot 10^{3}$ nl	20,7 nl	∞		

Expanded uncertainty of measurement:

$U = k \cdot u(V) = 2 \cdot 20{,}7 \text{ nl} \cong 41 \text{ nl}$

Complete measurement results for the volume:

$100,350 \cdot 10^{3}$ nl $\pm$ 41 nl

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The expanded uncertainty stated is the product of the standard uncertainty and the coverage factor $k = 2$. For a normal distribution it corresponds to a coverage probability of approx. 95%.

Remarks:

- The uncertainty contributions of weighing instrument reproducibility and weighing instrument resolution are listed twice as the weighing instrument is read twice, i.e. before and after dispensing. (Zero setting is equivalent to reading.)
  For demonstration, the measurement uncertainty budget contains a detailed list of uncertainty contributions assigned to the determination of the weighing value. The stated measurement uncertainty of the weighing instrument calibration is often that stated by the manufacturer or has been obtained from a previous measurement series. Splitting as shown in the table above can then be omitted but the measurement uncertainty must be taken into account twice, as **two** readings are carried out.

- This second method was used for thermometers, barometers and hygrometers. But only **one** reading is performed, and the uncertainty of measurement must be considered only once.

- Some of the numerical values for the sensitivity coefficients depend on the volume. It is, therefore, **not possible** to use the values of this example for other volumes. A detailed description of the calculation of the sensitivity coefficients can be found in [12].

- The resolutions of thermometer and barometer are considerably smaller than the uncertainties given. Their contribution to the uncertainty is, therefore, neglected.

- Correction for evaporation was not carried out. Half the maximum amount of evaporation occurring during a measurement is, therefore, assumed to be the half width of a rectangular distribution from which an uncertainty is determined. This value can be reduced by suitable corrections.

- Contributions to the measurement uncertainty to be attributed to inexact knowledge of air pressure, air temperature and relative air humidity are so small that it is in some cases justifiable to use standard values instead of the measured values. This applies in particular to the relative air humidity. (For a rectangular distribution, an interval of ± 20 % for the determination of the relative air humidity results in the same uncertainty as an interval of ± 4 mK for the determination of the water temperature.)

- An uncertainty contribution from the conversion of water temperature into water density, using Table A or the respective formula, is small compared with the uncertainty stemming from the determination of the liquid temperature.

- For dispensing devices made of different materials (e.g. piston-operated pipettes), a volume expansion coefficient cannot be stated. A measurement carried out as close to the desired temperature as possible avoids this problem.

The uncertainty of measurement related to gravimetric volume determination (corresponds to u_G in equation (1); in the above example: 21 nl) must be compared with the experimental standard deviation of the measurement (corresponds to u_V in equation (1); in the example: 0,40 µl) . From equation (1), the value 399 nl results for u_D. The requirement $u_G < 1/3\ u_D$ thus is met, and the experimental standard deviation can be used with sufficient accuracy as a measure of the measurement uncertainty for the volumetric apparatus.