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Experimental study on the calibration of piston-operated pipettes with air cushion

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Deutscher Kalibrierdienst (DKD) – German Calibration Service

Since its foundation in 1977, the German Calibration Service has brought together calibration laboratories of industrial enterprises, research institutes, technical authorities, inspection and testing institutes. On 3rd May 2011, the German Calibration Service was reestablished as a technical body of PTB and accredited laboratories.

This body is known as Deutscher Kalibrierdienst (DKD for short) and is under the direction of PTB. The guidelines and guides developed by DKD represent the state of the art in the respective areas of technical expertise and can be used by the Deutsche Akkreditierungsstelle GmbH (the German accreditation body – DAkkS) for the accreditation of calibration laboratories.

The accredited calibration laboratories are now accredited and supervised by DAkkS as legal successor to the DKD. They carry out calibrations of measuring instruments and measuring standards for the measurands and measuring ranges defined during accreditation. The calibration certificates issued by these laboratories prove the traceability to national standards as required by the family of standards DIN EN ISO 9000 and DIN EN ISO/IEC 17025.

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Foreword

DKD expert reports aim to provide background information and references in connection with other DKD documents as, for example, the DKD guidelines. In some cases, they may even go far beyond these documents. They do not replace the original DKD documents but do provide a lot of supplementary information worth knowing. The expert reports do not necessarily reflect the views of the DKD's Management Board or Technical Committees in all details.

DKD expert reports are intended to present significant aspects from the field of calibration. Through publication by the DKD they are made available to the large community of calibration laboratories, both nationally and internationally.

This expert report has been approved by the DKD Managing Board and was drawn up by the following authors:

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Revision 1 of this expert report differs from the original version in the following aspects:

- Minor typographical errors have been corrected. The wording and content are unchanged.
- The layout and the information on copyright have been revised.
- The expert report now has a DOI.

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Abstract

Within the scope of an international pilot study on the "Calibration of piston-operated pipettes with air cushion", the influences on the measurement uncertainty budgets have been extensively analysed. This pilot study was carried out as a laboratory comparison between twelve calibration laboratories which are accredited according to ISO/IEC 17025:2005 [1], and one National Metrology Institute. As calibration objects, piston-operated pipettes from different manufacturers were used.

Starting from the comprehensive calibration results, statistical methods for the determination of the reference value and of the attributed measurement uncertainties were applied. The mean value of the measurement results was selected to define the reference value, establishing the basis for the comparability of the measurement results of the participants. The standard deviation was used as the measurement uncertainty of the reference value and was found to lie within the tolerance limits according to standard EN ISO 8655 [2] and the different manufacturers' specifications.

The evaluation of the measurement results led to a more accurate estimation of previously known measurement uncertainty contributions as well as to the identification of new contributions to the measurement uncertainty budget.

Derived from this, the calibration procedures and the measuring conditions were specified or determined to ensure national and international comparability.

The results are part of the newly elaborated DKD guideline DKD-R 8-1 [3] for the calibration of piston-operated pipettes with air cushion.

1 Introduction

So far, the calibration of single- and multi-channel piston-operated pipettes with air cushion has been carried out by the gravimetric method in accordance with EN ISO 8655.
This calibration procedure is nationally and internationally approved by accreditation bodies according to EN ISO/IEC 17025:2005.
Due to different perspectives on the influences on the measurement result and, thus, on the associated measurement uncertainties, comparability of the measurement results could be guaranteed only partially.
Furthermore, the design and construction of the piston-operated pipettes, the measuring instruments used, the environmental conditions, the geographic location during the measurements, and the activities of the operators have a considerable influence on the measurement results and have to be taken into account.

The objective of assuring nationally and internationally comparable measurement results is a fundamental basis for correct and relevant measurement, which is the precondition for an accreditation according to EN ISO/IEC 17025:2005. To live up to this claim, the Technical Subcommittee of DKD *Volume/Density* decided to carry out the pilot study "Calibration of piston-operated pipettes with air cushion" in the form of an interlaboratory comparison.
All the calibration laboratories from Germany (DKD/DAkkS) and Switzerland (SCS) which were accredited at the beginning of the study in 2009 agreed to participate.
Assuring the comparability of measuring quantities and procedures is also of great importance nationally and internationally for the quantity "volume".
Therefore, also the National Metrology Institute of Thailand (NIMT) and a calibration laboratory from the USA, accredited according to ISO/IEC 17025:2005 by the American accreditation body PJLA, took part in the pilot study.
DKD-K-06901 of ZMK -ANALYTIK- GmbH was authorized by the Technical Subcommittee to assume the function of the pilot laboratory.
As a result, the pilot study was to give evidence of the influences having a significant effect on the measurement results and measurement uncertainties during the calibration of piston-operated pipettes with air cushion.

2 Preparation and implementation of the pilot study

In the Technical Subcommittee *Volume/Density*, a metrological concept was agreed on by the participants. This metrological concept took into consideration all the experience gained by the manufacturers and calibration service providers over years. The participating manufacturers of piston-operated pipettes agreed to provide different piston-operated pipettes and pipette tips.

2.1 Selection of the calibration objects

The decision was made to test, in the first phase of the interlaboratory comparison, exclusively piston-operated pipettes with air cushion. The reason for this is the special importance of the air cushion during the calibration of piston-operated pipettes.
To prove the general validity of the obtained information, piston-operated pipettes from different manufacturers were used as calibration objects:

- Single-channel piston-operated pipettes with a fixed volume
- Single-channel piston-operated pipettes with a variable volume
- Multi-channel piston-operated pipettes

In this way, there were always representatives of the manufacturers available who could clarify any questions concerning the functioning of the devices. For the calibration, only original tips from the respective manufacturer were used. Table 1 gives an overview of the piston-operated pipettes which were used in the pilot study.

Table 1: Calibration objects

Type	***Manufacturer***	***Number of channels***	***Measuring range***
LTS Pipet-lite FL 1000	Rainin	1	1000 µl
Transferpette® S (fix)	BRAND	1	1000 µl
Reference 4900 2500 (fix)	Eppendorf	1	2500 µl
Finnpipette Digital	Thermo Electron	1	10 µl - 100 µl
MCP LTS L-8x200	Rainin	8	20 µl - 200 µl
Transferpette® S 8-Channel (variable)	BRAND	8	20 µl - 200 µl
Finnpipette Digital MCP 8	Thermo Electron	8	50 µl - 300 µl

2.2 Agreements on the implementation and documentation of the results

It was agreed that the calibrations should be carried out according to the gravimetric method, in accordance with EN ISO 8655. This is the method for which all participating calibration laboratories were accredited. During the entire period of the pilot study, no participating laboratory was allowed to carry out any adjustment of the pipettes.
The calibration was to be carried out according to the respective detailed accredited calibration procedure.

In each laboratory, the piston-operated pipettes were to be calibrated, if possible, by different operators, in order to obtain and evaluate measuring values from different operators.
To document the measurement results, the participants submitted the following documents to the pilot laboratory:

- Calibration certificates (ILAC MRA)
- Calibration reports/test reports

Furthermore, the following additional information was provided for the evaluation (if it was not included in the calibration certificate):

- Specification of technical standards and equipment/weighing equipment
- Environmental conditions (temperature, relative humidity, atmospheric pressure)
- Indication of the water temperature
- Altitude of the calibration laboratory
- Important observations during the calibration

2.3 Implementation of the interlaboratory comparison

The experimental study was implemented in agreement with the requirements of standard EN ISO/IEC 17043:2010 [4] for providers of proficiency tests. All mandatory agreements were documented in a task description which was initially sent to the participants as a draft in order to gather comments and proposals for modifications.

The fundamental approach of an interlaboratory comparison according to EN ISO/IEC 17043:2010 is shown in Figure 1.

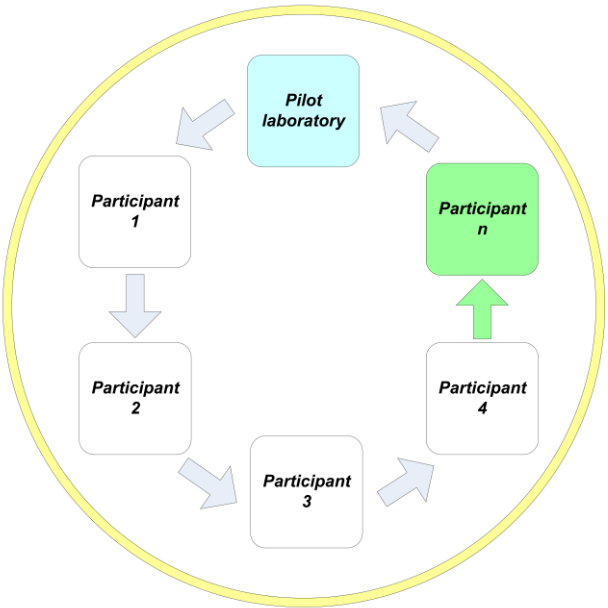

Figure 1: Implementation of an interlaboratory comparison according to EN ISO/IEC 17043:2010

The initial calibration of the piston-operated pipettes was carried out in the pilot laboratory. After that, the calibration objects were sent from one participating calibration laboratory to the next. A final calibration (back-measurement) took place again in the pilot laboratory.
The reference value for the individual piston-operated pipettes was to be defined jointly by the members of the working group once all the results were available.

3 General background and requirements

3.1 Definitions

Calibration certificate:
Calibration certificates document the results of calibrations, including the measurement uncertainty. In this guideline, the term "calibration certificate" applies to the following documents:

- Calibration certificates from calibration laboratories whose accreditation bodies have signed the ILAC MRA (see www.ilac.org)
- Calibration certificates from National Metrology Institutes with CMC entries (Appendix C of the CIPM MRA, see www.bipm.org)

The following terms have been taken from EN ISO 8655-1:

Piston-operated pipettes:
Piston-operated pipettes are volume measuring devices that are used to aspirate and dispense fixed or variable quantities of liquid. Single-channel piston-operated pipettes only have one piston/cylinder set. Multi-channel piston-operated pipettes have one piston/cylinder set for each channel. The same volume of liquid can be dispensed into several receptacles at the same time. A differentiation is made between piston-operated pipettes with or without air cushion (positive displacement pipettes).

Nominal volume:
The nominal volume (V_0) of a volume measuring device is the volume defined by the manufacturer to identify and specify the measuring range. For multi-channel piston-operated pipettes, the nominal volume is specified for each individual channel.

Useful volume range:
The useful volume range of a volume measuring device with a variable volume is a sub-range of the nominal volume. Within this sub-range, dispensing can be completed under observance of the maximum permissible errors defined in the international standard ISO 8655. The upper limit of the useful volume range is always the nominal volume. If not specified otherwise by the supplier, the lower limit is 10 % of the nominal volume.

Selected volume:
The selected volume V_S of a volume measuring device with a variable volume is the volume set by the user to dispense a selected volume from the useful volume range of a piston-operated device. In the case of volume measuring devices with a fixed volume, the selected volume is the nominal volume.

Volume of the air cushion (dead volume):
The volume of the air cushion (V_T) is the geometric space between the piston and the tip opening. The expansion of the air cushion volume is defined by the first stop of the piston in the pipette.

3.2 Applicable standards and regulations

EN ISO 8655 Parts 1, 2, 6	Piston-operated volumetric apparatus
ISO/TR 20461	Determination of uncertainty for volume measurements made using the gravimetric method, November 2000 and ISO/TR 20461 Technical Corrigendum 1, December 2008
JCGM 100: 2008	Evaluation of measurement data – Guide to the expression of uncertainty in measurement, September 2008
EURAMET/cg-18 Version 3.0	Guidelines on the Calibration of Non-automatic Weighing Instruments, March 2011
EURAMET/cg-19 Version 2.1	Guidelines on the determination of uncertainty in gravimetric volume calibration, March 2012
ISO 3696	Water for analytical laboratory use, June 1991
DAkkS-DKD-3	Information on measurement uncertainty with calibrations, 2010

3.3 Abbreviations and symbols

Table 2: Abbreviations and symbols

Abbreviations / symbols	Explanation
a_0 to a_4	Constants (ITS-90 temperature scale) for calculating the water density
c	Sensitivity coefficient
s	Standard deviation
CV	Random error as variation coefficient indicated in percent
e_S	Systematic error
g	Gravitational acceleration

h_W	Lifting height of the liquid column in the pipette tip
i	Continuous index
k_1 to k_3	Constants (ITS-90 temperature scale) for calculating the air density
m	Mass of the test liquid (corresponding to the difference of the balance readings)
m_E	Loss of mass due to evaporation
n	Number of individual measurements
p_L	Atmospheric pressure
T_W	Temperature of the test liquid
T_L	Air temperature during weighing
T_{L0}	273.15 K
T_M	Temperature of the piston-operated pipette during measurement
T_{M20}	Piston-operated pipette reference temperature of 20 °C
u	Standard measurement uncertainty
U	Expanded measurement uncertainty (k = 2); value of the measurand with a probability of 95 % in the attributed interval of values
V_0	Nominal volume
V_S	Selected volume
V_{20}	Volume at 20 °C reference temperature
V_T	Volume of the air cushion (dead volume)
x_i	Measuring value of a participant
X_{Ref}	Reference value
Z	Correction factor describing the relationship between the mass which has been determined during weighing, and the volume
ρ_L	Air density
ρ_W	Density of the water used as a test liquid
ρ_G	Density of the standard weights used to calibrate the balance (equal to 8000 kg/m^3)
ϕ	Relative humidity
γ	Cubic coefficient of expansion of the material from which the pipette is made

3.4 Units of measurement

Table 3: Units of measurement

Units of measurement	Explanation
µl	Microlitre
ml	Millilitre
g	Gram
mg	Milligram
K	Kelvin
°C	Degrees Celsius
hPa	Hectopascal
%	Percent of relative humidity
g/cm³	Gram per cubic centimetre
µl/mg	Microlitre per milligram

4 Evaluation of the results of the pilot study

4.1 Graphical representation of the measurement results

The measurements within the scope of the pilot study were carried out from 06/2009 to 09/2010. The predetermined time schedule as well as the requirements for the documentation of the results were complied with.

Due to the high number of accredited calibration laboratories participating, a very extensive data pool was available so that significant influences could be determined. This comprehensive data collection was recorded in tabular form. After that, a graphical representation of the measurement results with the associated expanded measurement uncertainties ($k = 2$) was created.

Selected examples of this are shown graphically for different piston pipettes in Figs. 2, 3 and 4. In the graphical evaluation, the tolerance limits of EN ISO 8655 and the specifications of the manufacturers were taken into account.

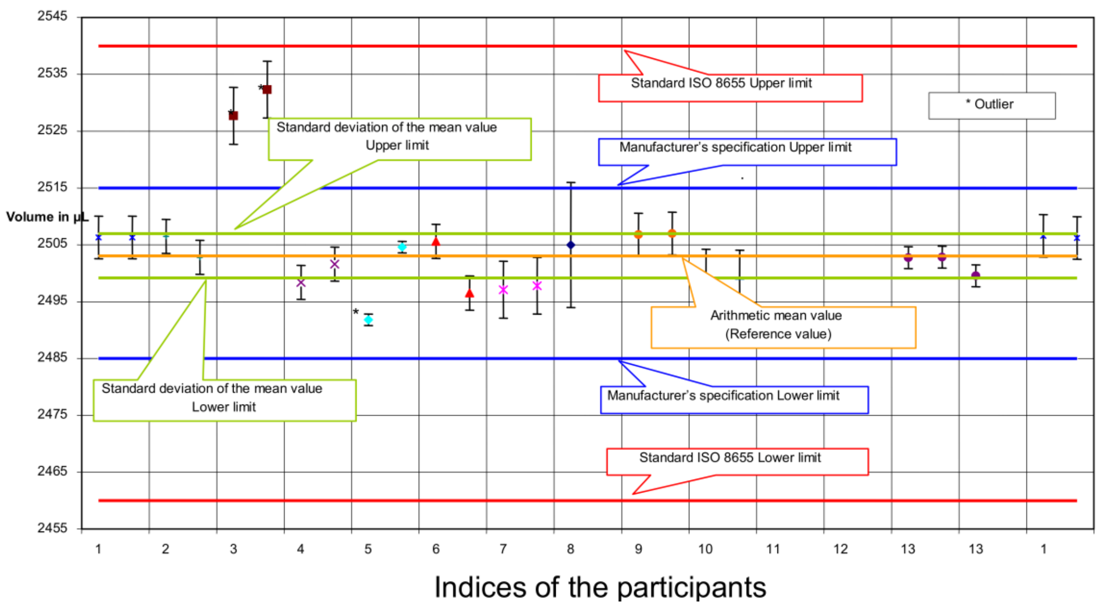

Figure 2: Graphical representation of the measurement results for a single-channel piston-operated pipette with a fixed volume (Eppendorf Reference 2500 µl)

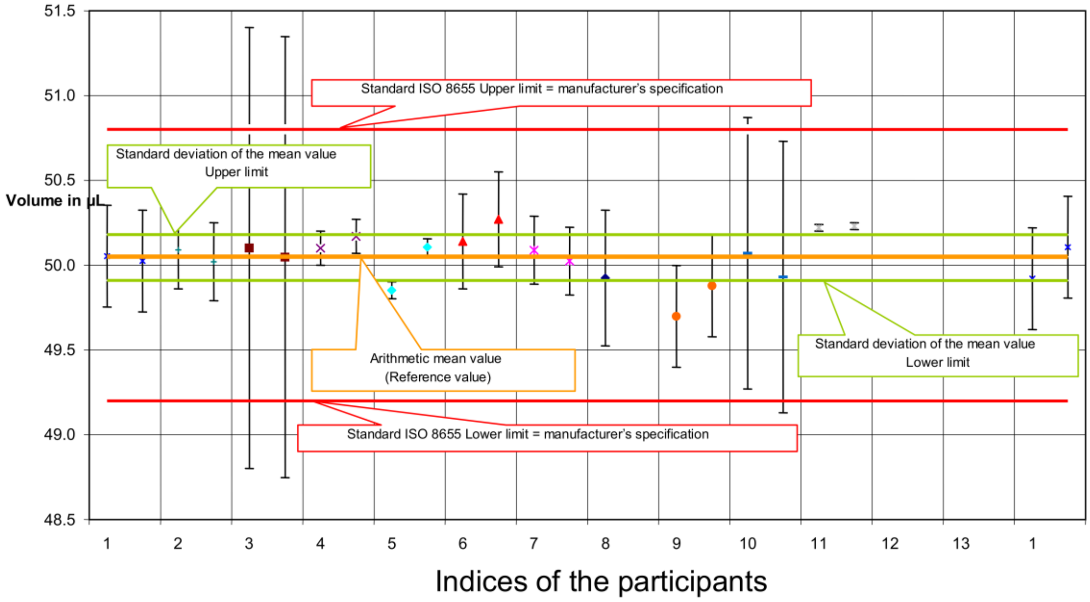

Figure 3: Graphical representation of the measurement results for a single-channel piston-operated pipette with a variable volume (Finnpipette Digital 10 µl - 100 µl; tested volume 50 µl)

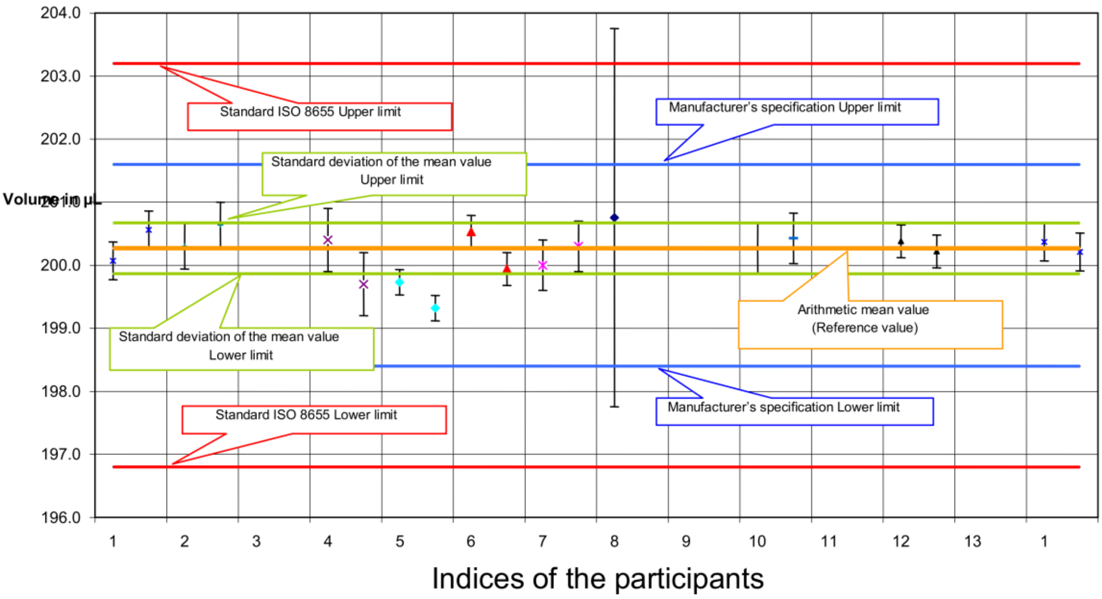

Figure 4: Graphical representation of the measurement results for a multi-channel piston-operated pipette with a variable volume (Transferpette S 20 µl - 200 µl; channel 1; tested volume 200 µl)

4.2 Determination of the reference value and of its measurement uncertainty

In the following section, it will be shown in which way the determination of the reference value and of its measurement uncertainty was carried out. The selection of the reference value and of the measurement uncertainty is of great importance for the analysis of comparison measurements/interlaboratory comparisons. The reference value is decisive for the comparability of measuring values and is the basis for correct measurement.

To determine the reference value of a comparison measurement, different methods according to ISO 13528:2009 [5] are available. These methods will be outlined in the following:

- Mean value of the pilot laboratory (mean value obtained from the initial calibration, intermediate measurement, if available, and back-measurement)

$$X_{\text{Ref}} = \frac{\sum_{i=1}^{n} x_{i,\text{Pilot}}}{n} \quad (1)$$

- Mean value obtained from the measured values of the participants

$$X_{\text{Ref}} = \frac{\sum_{i=1}^{n} x_{i,\text{Lab}}}{n} \quad (2)$$

- Weighted mean value obtained from the measured values of the participants (as a function of the measurement uncertainty)

$$X_{\text{Ref}} = \frac{\sum_{i=1}^{n} \frac{x_{i,\text{Lab}}}{u_i^2}}{\sum_{i=1}^{n} \frac{1}{u_i^2}} \quad (3)$$

- Reference value obtained from a superordinated interlaboratory comparison (key comparison or RMO comparison)
- The reference value is provided by an NMI which has the respective CMC entries in the BIPM data base for this measuring quantity and a correspondingly small measurement uncertainty

The method to determine the reference value is selected as a function of the measuring quantity and of the applied calibration procedure. It is agreed between the participants of a comparison measurement before the comparison measurement is started.

As, for the calibration of piston-operated pipettes, no primary or absolute method is available which would allow the direct use of a reference value, the arithmetic mean value (unweighted) was used for the pilot study as the reference value of all the participants and of the pilot laboratory. One of the reasons for selecting this method was that all participating laboratories are accredited according to EN ISO/IEC 17025:2005 and thus work with the same calibration procedure according to EN ISO 8655. This means that the comparability of the measurement results is given. Distinct outliers were excluded.

In a second step, the uncertainty of the reference value was determined. According to ISO 13528, the following methods are, for example, available for this:

- If each single laboratory out of the n participating laboratories reports a measurement xi of the calibration object together with the standard uncertainty of the measurement ui and the reference value X_{Ref} is determined as the mean value, the standard measurement uncertainty of the reference value X_{Ref} is estimated as follows:

$$u_{\text{Ref}} = \frac{1{,}25}{n} \cdot \sqrt{\sum_{i=1}^{n} u_i^2} \qquad (4)$$

- If the participating laboratories do not state any standard uncertainties, the standard measurement uncertainty of the reference value has to be estimated as follows:

$$u_{\text{Ref}} = \frac{1{,}25}{\sqrt{n}} \cdot s \qquad (5)$$

whereby s is the standard deviation of the measurement results.

- If the reference value is calculated from the weighted mean value of the measuring values of the participants, the standard uncertainty of the reference value is derived as follows:

$$u_{\text{Ref}} = \sqrt{\sum_{i=1}^{n} \left(\frac{\partial X_{\text{Ref}}}{\partial x_{i,\text{Lab}}}\right) \cdot u_i^2} \qquad (6)$$

All participants of the pilot study calculated their measurement uncertainty according to Guideline ISO/TR 20461 [6]. The quantitative estimation of some uncertainty contributions was, however, partly too optimistic or erred on the side of caution. This is why a very wide range of measurement uncertainties was reported by the participants. To evaluate the pilot study, the standard deviation of the measurement values of the participants was therefore used as the uncertainty for the reference value.

As can be seen in the graphical evaluations (Figures 2, 3 and 4), almost all measurement values are within the tolerance of the reference value. This shows that the methods selected for the determination of the reference value (arithmetic mean value) and the assigned measurement uncertainty (standard deviation) agree well with the measuring values.
Defined reference values for measurement procedures for comparison measurements / interlaboratory comparisons are the precondition for the metrologically correct interpretation of data.

Furthermore, it is acceptable, from a technical point of view, to use the standard deviation as a measure for measurement uncertainties to be stated also for the calibration of piston-operated pipettes as they, too, lie within the error limits according to EN ISO 8655 and the more limited manufacturer's specifications. In practice, compliance with the manufacturer's specification is increasingly gaining in importance as this is a quality criterion for the implementation of the processes by the users.

5 Influences on the measurement uncertainty budget

5.1 Cause-effect-diagram

After the extensive measurement values and the graphical representations which had been determined within the scope of the pilot study had been processed, all those contributions having an influence on the measurement result were evaluated. The essential influences are shown in the cause-effect-diagram.

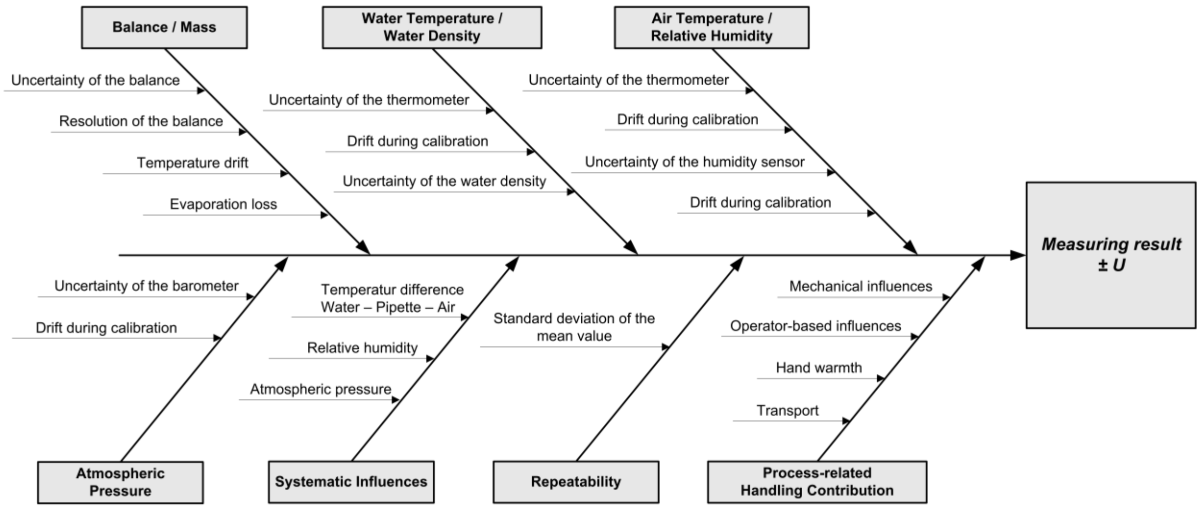

Figure 5: Cause-effect-diagram for the calibration of different piston-operated pipettes with air cushion

5.2 Re-evaluation of uncertainty contributions

The cause-effect-diagram shows the measuring conditions or, respectively, the uncertainty contributions which were taken into account during the establishment of the measurement uncertainty budget.

The measuring conditions and the uncertainty contributions were re-evaluated and defined:

- Uncertainty contributions of the balance
- Air temperature and relative humidity (environmental conditions) / water temperature
- Water temperature / water density
- Atmospheric pressure
- Systematic influences (influences of the environmental conditions on the air cushion of the piston-operated pipette)
- Repeatability
- Process-related handling contribution (mechanical influences, operator, etc.)

The most important uncertainty components, in turn, consist of single contributions. The measurement conditions and the uncertainty contributions were specified, re-evaluated and defined within the scope of the evaluation of the extensive measurement values.

5.2.1 Uncertainty contributions of the balance

The calibration certificate (according to EURAMET/cg-18 [7]) is the basis for the consideration of the measurement uncertainty for the balance used.
It is necessary to take the readability/resolution of the balance into account twice, as balance taring is an integral step in weighing.
The influence of the environmental conditions (temperature drift) according to the manufacturer's information must also be taken into account.
The drift behaviour of the balance due to aging or, respectively, wear should be monitored continuously and determined by intermediate checks or recalibration.
This influence can additionally be taken into account, on the basis of long-term observations. As, during the dispensing process with piston-operated pipettes, free liquid surfaces occur, a contribution for the evaporation loss should be taken into account in the measurement uncertainty budget.

5.2.2 Air temperature and relative humidity (environmental conditions), water temperature and water density

Air temperature/relative humidity
During the calibration of piston-operated pipettes with air cushion, the environmental conditions "air temperature" and "relative humidity" are realized by establishing and complying with pre-defined parameters. The measuring data of the environmental conditions are recorded and documented during the calibration by calibrated temperature and humidity sensors.

The fluctuations of the air temperature during the calibration were usually between (20 to 23) °C ± 0.5 K.

The relative humidity during the calibration was determined to be > (50 to 65) % ± 5 %.

In the measurement uncertainty budget, both the fluctuations of the environmental conditions during the calibration and the uncertainty of the measuring devices are taken into account.

Quality of the water/Density of the water
For the calibration, deionized water of quality 3 according to ISO 3696 [8] is used, with an electrolytic conductivity of < 5 µS/cm. The uncertainty of the calculation of the water density is estimated according to TANAKA [9] to $10 \cdot 10^{-6}$, as the exact isotopic ratio and the gas content are not known. The water density is part of the calculation of the volume of the test liquid.

Water temperature/Air temperature
It must be ensured that the water temperature approaches the air temperature, i.e. the temperature difference between the air and the water is essential for the quality of the measurements with small standard deviations.
It should be ensured, as a matter of principle, that the temperature difference between the air and the water is very small. As a result of the pilot study it could be proven that for smaller measurement uncertainties (participation in interlaboratory comparisons), the difference between the air temperature and the water temperature should be < 0.2 K.
However, a temperature difference between the air and the water of ≤ 0.5 K must strictly be observed in order to obtain comparable measurement results.

5.2.3 Atmospheric pressure

The atmospheric pressure has an influence on the volume in the pipette and is therefore a necessary measuring quantity for the calculation of the air density and, thus, of the volume. For the measurement of the atmospheric pressure, a precision barometer is used. The fluctuations during the calibration should not exceed 1 hPa.
In the measurement uncertainty budget, the fluctuations of the atmospheric pressure during the calibration as well as the uncertainty of the precision barometer are taken into account.

5.2.4 Observation of the systemic influences of the piston-operated pipettes with air cushion during the calibration

Volume dispensing in a piston-operated pipette with air cushion is a thermodynamic process. It begins when the pipette tip is immersed into the water and ends when the pipette tip is removed (separation of the liquid column). The influences of the piston-operated pipette depend, in particular, on the size of the air cushion and on the lifting height of the liquid column in the pipette tip. For that reason, the following influences are taken into account in the measurement uncertainty budget:

- Temperature differences between the water, the pipette and the air
- Relative humidity
- Atmospheric pressure

In connection with the construction of the system *"piston-operated pipette – pipette tip"*, the conditions have to be reconsidered. These systematic influences of piston-operated pipettes with air cushion were investigated in detail in [10] and [11].

5.2.4.1 Temperature differences between the water, the pipette and the air

The temperature differences between the water, the pipette and the air in the pipette system/pipette tip during the calibration have a great influence on the dispensed volume.
This important conclusion from the pilot study means that a small temperature difference between the water, the pipette and the air can be ensured by a sufficiently long equilibration time.

5.2.4.2 Relative humidity

The relative humidity has an additional system-related influence on the measurement result as the evaporation of the test liquid depends directly on the relative humidity of the environment. Already during the aspiration process, an evaporation of the liquid takes place and the smallest evaporated amounts of liquid lead to a large volume displacement in the air cushion of the piston-operated pipette [10].

It could be proven that the relative humidity of the environment should be in the range of (50 ± 5) % during the calibration.

5.2.4.3 Atmospheric pressure/Altitude

The evaluation of the pilot study has shown that the calibration of piston-operated pipettes with air cushion at different altitudes has a significant influence on the measurement results.
From thermodynamics, it is derived that at high altitudes, the volume distinctly decreases due to the low air density. For that reason, corrections for the altitude [6] have to be made in order to achieve the comparability of the calibration results.

The influence of the altitude on the volume result of a piston-operated pipette with air cushion was experimentally investigated by Mr Christoph Spälti, Spaelti-TS AG, at different altitudes [12]. The investigations have shown that the determined measuring value can be corrected to the same altitude as the pilot laboratory under consideration of the present measurement uncertainties. The equation for the correction of the volume and the input quantities necessary for that are explained in the publication of BRAND GMBH + CO KG and the *Fraunhofer-Institut für Silicatforschung* [10].

The investigation results with regard to the influence of the altitude are summarized in the study "The influence of altitude on the volume result of a piston pipette with air cushion" and are therefore an additional important contribution to the accomplished pilot study.

For the general meteorological fluctuations of the atmospheric pressure, a contribution of ± 20 hPa is taken into account in the measurement uncertainty budget.

If the specified conditions (Sections 5.2.2, 5.2.3 and 5.2.4) cannot be realized, the respective contributions must be estimated to a correspondingly higher value in the measurement uncertainty budget.

5.2.5 Process-related handling contribution

The *"process-related handling contribution"* is a new measurement uncertainty contribution. It results directly from the evaluation of the extensive measurement values of the accomplished pilot study. This contribution covers the errors occurring during the calibration of piston-operated pipettes with air cushion and is taken into account in the measurement uncertainty budget as follows:

- for single-channel piston-operated pipettes with a fixed volume: with 0.07 % of the nominal volume; and
- for single-channel piston-operated pipettes with a variable volume and for multi-channel piston-operated pipettes: with 0.1 % of the nominal volume.

Various influences contribute to the process-related handling contribution; the most important influences are:

- the calibration procedure, e.g. tip preconditioning, tip replacement, and others
- mechanical influences due to the construction of the calibration object
- operator-based influences, e.g. operating force, pipetting rhythm
- hand warmth
- transport

5.3 Measurement uncertainty budget

As a result of the new approach for the determination of the measurement uncertainty for the calibration of piston-operated pipettes with air cushion, a measurement uncertainty budget is shown in Table 4 for a piston-operated pipette with a variable volume and a nominal volume of 100 µl.

Quantity X_i	Estimate x_i	Half width of distribution a	Probability distribution $P(x_i)$	Divider k	Standard uncertainty $u(x_i)$	Sensitivity coefficient c_i	Uncertainty contribution $u_i(y)$
Balance / Mass							
Uncertainty of balance	0 mg	25 µg	normal	2	12.500 µg	0.001 µL/µg	0.0125 µL
Resolution of balance (with load)	100.059 mg	0.5 µg	rectangular	$\sqrt{3}$	0.289 µg	0.001 µL/µg	0.0003 µL
Resolution of balance (without load)	0.000 mg	0.5 µg	rectangular	$\sqrt{3}$	0.289 µg	0.001 µL/µg	0.0003 µL
Temperature drift	0 mg	0.2 K	rectangular	$\sqrt{3}$	0.115 K	0.0001 µL/K	0.0000 µL
Evaporation loss	0 mg	15 µg	rectangular	$\sqrt{3}$	8.660 µg	0.001 µL/µg	0.0087 µL
Water temperature / density							
Uncertainty of thermometer	21.7 °C	0.012 K	normal	2	0.006 K	0.021 µL/K	0.0001 µL
Drift during calibration	0 °C	0.2 K	rectangular	$\sqrt{3}$	0.115 K	0.021 µL/K	0.0024 µL
Uncertainty of water density	997.84 kg/m³	10 ppm	rectangular	$\sqrt{3}$	0.00001 mg/µL	-100 µL²/mg	-0.0006 µL
Air temperature							
Uncertainty of thermometer	21.8 °C	0.13 K	normal	2	0.065 K	0.00045 µL/K	0.0000 µL
Drift during calibration	0 °C	0.2 K	rectangular	$\sqrt{3}$	0.115 K	0.00045 µL/K	0.0001 µL
Atmospheric pressure							
Uncertainty barometer	1009 hPa	0.05 hPa	normal	2	0.025 hPa	0.00012 µL/hPa	0.0000 µL
Drift during calibration	0 hPa	1 hPa	rectangular	$\sqrt{3}$	0.577 hPa	0.00012 µL/hPa	0.0001 µL
Relative humidity							
Uncertainty humidity sensor	53 % RH	0.6 % RH	normal	2	0.300 % RH	0.00001 µL/% RH	0.0000 µL
Drift during calibration	0 % RH	5 % RH	rectangular	$\sqrt{3}$	2.887 % RH	0.00001 µL/% RH	0.0000 µL
Temp. difference water-pipette-air	0.0 °C	0.2 K	rectangular	$\sqrt{3}$	0.115 K	0.22 µL/K	0.0254 µL
Relative humidity	53 % RH	5 % RH	rectangular	$\sqrt{3}$	2.887 % RH	0.007 µL/% RH	0.0202 µL
Atmospheric pressure	1009 hPa	20 hPa	triangular	$\sqrt{6}$	8.165 hPa	0.0012 µL/hPa	0.0098 µL
Repeatability	0 mg	0.17 µL	normal	$\sqrt{10}$	0.053 µL	1	0.0527 µL
Process-related handling contribution	0 mg	0.1 µL	rectangular	$\sqrt{3}$	0.058 µL	1	0.0577 µL
Y (Volume)	100.38 µL					$u(y)$ =	0.087 µL
						$U(y)$ =	**0.20 µL**
						$w(y)$ =	0.09 %
						$W(y)$ =	**0.20 %**

Table 4: Example of a measurement uncertainty budget for a piston-operated pipette with a variable volume (nominal volume 100 µl)

As can be seen in the measurement uncertainty budget, the following contributions have a decisive influence on the measurement uncertainty during the calibration of piston-operated pipettes with air cushion:

- influence of the environmental conditions in connection with the construction of the piston-operated pipettes with air cushion
- repeatability of the measuring values
- process-related handling contribution (mechanical influences, operator, etc.)

As an important additional result of the pilot study it could be ascertained that the process-related influences and the influences of the environmental conditions are the determining contributions for the measurement uncertainty budget.

6 Summary and outlook

Based on the extensive measurement results obtained in the pilot study, it was possible to re-evaluate several contributions and to identify new contributions. In addition, process-related measuring conditions and approaches could be defined in a new way or, respectively, in a more detailed way. The findings obtained were the basis for the elaboration of the DKD guideline DKD-R 8-1 *"Calibration of piston-operated pipettes with air cushion"* which has established the basis for the further work of the accredited calibration laboratories in the German Federal Republic since its coming into force.

It is intended to present the English version to EURAMET. This will make it possible for an even wider circle of calibration laboratories to make use of the knowledge and findings and will lead, in this way, to better national and international comparability of the measuring values during the calibration of piston-operated pipettes with air cushion.

The work of the Technical Subcommittee of DKD *Volume/Density* will be continued. In a second period of investigation, selected pipettes will now be investigated which operate according to the principle of positive displacement. Many findings obtained from the pilot study on piston-operated pipettes with air cushion will establish the basis for these new metrological investigations.

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