

Physikalisch- Technische Bundesanstalt




Expert Report DKD-E 8-4

Tip replacement in the calibration of
piston-operated pipettes – economic
and environmental implications

Edition 10/2023

<https://doi.org/10.7795/550.20231106>



	<p>Tip replacement in the calibration of piston-operated pipettes – economic and environmental implications</p> <p>https://doi.org/10.7795/550.20231106</p>	DKD-E 8-4	
		Edition:	10/2023
		Revision:	0
		Page:	2 / 13

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.

Contact:

Physikalisch-Technische Bundesanstalt (PTB)

DKD Executive Office


Bundesallee 100 38116 Braunschweig

P.O. Box 33 45 38023 Braunschweig

GERMANY

Telephone: +49 531 592-8021

Internet: <https://www.ptb.de/cms/en/metrological-services/dkd.html>

	<p>Tip replacement in the calibration of piston-operated pipettes – economic and environmental implications</p> <p>https://doi.org/10.7795/550.20231106</p>	DKD-E 8-4	
		Edition:	10/2023
		Revision:	0
		Page:	3 / 13

Suggestion for the citation of sources:

Expert report DKD-E 8-4 Tip replacement in the calibration of piston-operated pipettes – economic and environmental implications, Edition 10/2023, Revision 0, Physikalisch-Technische Bundesanstalt, Braunschweig and Berlin. DOI: 10.7795/550.20231106


This document and all parts contained therein are protected by copyright and are subject to the Creative Commons user license CC by-nc-nd 3.0 (<http://creativecommons.org/licenses/by-nc-nd/3.0/de/>). In this context, “non-commercial” (NC) means that the work may not be disseminated or made publicly accessible for revenue-generating purposes. The commercial use of its contents in calibration laboratories is explicitly allowed.



Authors:

Dr. Olaf Schnelle-Werner, ZMK & ANALYTIK GmbH, Wolfen
 Dr. Ulrich Breuel, ZMK & ANALYTIK GmbH, Wolfen
 Elke Winzer, ZMK & ANALYTIK GmbH, Wolfen

Published by the Physikalisch-Technische Bundesanstalt (PTB) for the German Calibration Service (DKD) as result of the cooperation between PTB and DKD's Technical Committee *Chemical Measurands and Material Properties*.

	<p>Tip replacement in the calibration of piston-operated pipettes – economic and environmental implications</p> <p>https://doi.org/10.7795/550.20231106</p>	DKD-E 8-4	
		Edition:	10/2023
		Revision:	0
		Page:	4 / 13

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.



	<p>Tip replacement in the calibration of piston-operated pipettes – economic and environmental implications</p> <p>https://doi.org/10.7795/550.20231106</p>	DKD-E 8-4	
		Edition:	10/2023
		Revision:	0
		Page:	5 / 13

Table of contents

1	Background	6
2	Carrying out the investigations	6
3	Performing the measurements	6
4	Calibration objects	7
5	Calibration results	7
6	Evaluation of the results	11
7	Economic and environmental implications	11
7.1	Economic consequences	12
7.2	Environmental consequences	12
8	Bibliography	12

Abbreviations / Explanation of terms used

DAkKS	Deutsche Akkreditierungsstelle GmbH (the German accreditation body)
DIN	Deutsches Institut für Normung
DKD	Deutscher Kalibrierdienst (German Calibration Service)
DKD-R	Calibration guideline of DKD
EN	European standard
IEC	International Electrotechnical Commission
ISO	International Organization for Standardization
PTB	Physikalisch-Technische Bundesanstalt
ZMK	Zentrum für Messen und Kalibrieren & ANALYTIK GmbH

	<p>Tip replacement in the calibration of piston-operated pipettes – economic and environmental implications</p> <p>https://doi.org/10.7795/550.20231106</p>	DKD-E 8-4	
		Edition:	10/2023
		Revision:	0
		Page:	6 / 13

1 Background

According to the new version of ISO 8655-6 [1], a tip change must be performed when calibrating piston-operated pipettes with air cushion.

The requirements for tip replacement are given in chapter 8.3.2 of ISO 8655-6.

The necessity is explained by the fact that the tip change would reveal the use of damaged or incorrectly manufactured tips. The requirement also applies to direct displacement piston-operated pipettes.

For a series of 10 repeated measurements, at least 2 tips should be used. The change should therefore be made after 5 measurements. This results in a scheme of 2 x 5 as a minimum requirement. A scheme with more than one change is not excluded by the standard according to the authors' interpretation.

To verify the influence of the tip change, ZMK has carried out repeated investigations.

2 Carrying out the investigations

For the tests, 10 piston-operated pipettes with air cushion were selected according to the following criteria:

- single channel piston pipettes (variable and fixed volume)
- customer devices (no reference devices from ZMK, because they are subject to special maintenance)
- different manufacturers
- different measuring ranges

The pipettes were first calibrated as part of customer orders. In accordance with DKD-R 8-1 [2], the tips were not changed. These results, as commissioned by the customers, were documented in DAkkS calibration certificates.

In addition, the pipettes were then recalibrated, and a tip change was performed. The results of these calibrations were not made available to the customers.

The results of both calibrations were compared by calculating the E_n value.

3 Performing the measurements

Calibrations without tip change were performed in the following steps:

1. attaching the tip
2. moistening of the air cushion, 5 times
3. recording of 10 measurement values
4. discarding of the tip

The measurements with tip change according to the scheme 2 x 5 included the following steps:

1. mounting the tip
2. moistening of the air cushion, 5 times
3. recording of 5 measurement values
4. discarding of the tip
5. attaching (mounting) of a new tip
6. moistening of the air cushion, 5 times
7. recording of 5 measurement values
8. discarding of the tip

4 Calibration objects

The following piston-operated pipettes with air cushion from different manufacturers were used for the investigations:

- Eppendorf Reference blue, 500 µl, serial number 4103124
- Eppendorf Reference 2 red, 250 µl - 2500 µl, serial number R34938C
- Eppendorf Reference grey/yellow, 10 µl, serial number 2365997
- Eppendorf Research plus, 10 µl - 100 µl, serial number K24886J
- Socorex Calibra 822, 100 µl - 1000 µl, serial number 18041035
- BRAND Transferpette, 500 µl - 5000 µl, serial number 09J48760
- Rainin Pipet-Lite, 100 µl - 1000 µl, serial number H0100141A
- BRAND Transferpette S, 1000 µl, serial number 10F88916
- Thermo Electron Finnpipette, 10 µl - 100 µl, serial number DH21843
- HTL CLINIPET + CP5, 5 µl, serial number 922040055

Only original tips from the respective pipette manufacturers were used for testing.

5 Calibration results

The following tables 1 to 10 show the results of the calibrations, including the E_n value.

Volume without tip change	Expanded uncertainty ($k = 2$) without tip change	Volume with tip change	Expanded uncertainty ($k = 2$) with tip change	E_n value
in µl	in µl	in µl	in µl	
499.76	0.60	499.74	0.60	0.02

Table 1: Results for Eppendorf Reference blue, 500 µl, serial number 4103124

Volume without tip change in μl	Expanded uncertainty ($k = 2$) without tip change in μl	Volume with tip change in μl	Expanded uncertainty ($k = 2$) with tip change in μl	E_n value
254.71	1.88	254.68	1.88	0.01
1252.5	2.8	1252.5	2.8	0.00
2499.3	3.8	2499.2	3.8	0.02

Table 2: Results for Eppendorf Reference 2 red, 250 μl - 2500 μl , serial number R34938C

Volume without tip change in μl	Expanded uncertainty ($k = 2$) without tip change in μl	Volume with tip change in μl	Expanded uncertainty ($k = 2$) with tip change in μl	E_n value
10.08	0.03	10.08	0.03	0.02

Table 3: Results for Eppendorf Reference grey/yellow, 10 μl , serial number 2365997

Volume without tip change in μl	Expanded uncertainty ($k = 2$) without tip change in μl	Volume with tip change in μl	Expanded uncertainty ($k = 2$) with tip change in μl	E_n value
10.059	0.075	10.063	0.075	0.04
50.055	0.110	50.054	0.110	0.01
100.281	0.150	100.273	0.150	0.04

Table 4: Results for Eppendorf Research plus, 10 μl - 100 μl , serial number K24886J

Volume without tip change in μl	Expanded uncertainty ($k = 2$) without tip change in μl	Volume with tip change in μl	Expanded uncertainty ($k = 2$) with tip change in μl	E_n value
101.00	0.75	101.29	0.75	0.27
497.8	1.1	498.2	1.1	0.31
996.6	1.5	996.6	1.5	0.03

Table 5: Results for Socorex Calibra 822, 100 μl - 1000 μl , serial number 18041035

Volume without tip change in μl	Expanded uncertainty ($k = 2$) without tip change in μl	Volume with tip change in μl	Expanded uncertainty ($k = 2$) with tip change in μl	E_n value
489.08	3.75	491.37	3.75	0.43
2492.8	5.5	2491.2	5.5	0.21
5012.3	7.5	5010.4	7.5	0.18

Table 6: Results for BRAND Transferpette, 500 μl - 5000 μl , serial number 09J48760

Volume without tip change in μl	Expanded uncertainty ($k = 2$) without tip change in μl	Volume with tip change in μl	Expanded uncertainty ($k = 2$) with tip change in μl	E_n value
102.186	0.750	102.176	0.750	0.01
498.47	1.10	498.51	1.10	0.03
997.90	1.50	997.89	1.50	0.00

Table 7: Results for Rainin Pipet-Lite, 100 μl - 1000 μl , serial number H0100141A

Volume without tip change in μl	Expanded uncertainty ($k = 2$) without tip change in μl	Volume with tip change in μl	Expanded uncertainty ($k = 2$) with tip change in μl	E_n value
999.99	1.20	999.96	1.20	0.02


Table 8: Results for BRAND Transferpette S, 1000 μl , serial number 10F88916

Volume without tip change in μl	Expanded uncertainty ($k = 2$) without tip change in μl	Volume with tip change in μl	Expanded uncertainty ($k = 2$) with tip change in μl	E_n value
10.191	0.075	10.191	0.075	0.00
100.375	0.150	100.375	0.150	0.00

Table 9: Results for Thermo Electron Finnpiquette, 10 μl - 100 μl , serial number DH21843

Volume without tip change in μl	Expanded uncertainty ($k = 2$) without tip change in μl	Volume with tip change in μl	Expanded uncertainty ($k = 2$) with tip change in μl	E_n value
5.051	0.038	5.052	0.038	0.02

Table 10: Results for HTL CLINIPET + CP5, 5 μl , serial number 922040055

	Tip replacement in the calibration of piston-operated pipettes – economic and environmental implications https://doi.org/10.7795/550.20231106	DKD-E 8-4	
		Edition:	10/2023
		Revision:	0
		Page:	11 / 13

6 Evaluation of the results

The absolute values of E_n are significantly smaller than 1. This applies to pipettes from different manufacturers and with different measuring ranges (also < 100 µl) as well as to pipettes from daily use at customers (no reference pipettes).

An influence of the tip change could therefore not be demonstrated.

This confirms the practice of pipette calibration according to DKD-R 8-1:

7.2 Pipette tips – Accessories for dispensing

Pipette tips which are attached to the pipette shaft are used for volume dispensing with piston-operated pipettes. Only unused pipette tips approved by the manufacturer may be used. Like the piston-operated pipettes, the pipette tips have to be stored in the measuring room for at least two hours before starting calibration.


According to EN ISO 8655-2, a tip replacement is recommended after each individual measurement. However, deviations from this rule are allowed. According to this guideline, a pipette can be calibrated with one pipette tip per channel. However, the air cushion still needs to be pre-wet five times at the start of the calibration. Pre-wetting should also be conducted when the volume is changed (setting of a new test volume). If residues remain in the tip, the tip has to be replaced categorically.

If a tip is to be replaced, the new pipette tip must be pre-wet five times as well.

7 Economic and environmental implications

The issue of tip replacement continues to be discussed in technical committees. There is no consensus on the necessity of this measure. There are certainly cases where a tip change is appropriate and necessary to obtain correct calibration results. The investigations carried out have not shown any significant influence.

Apart from the technical aspect, the authors are of the opinion that there are more far-reaching consequences, which are referred to in the conclusion. These are of an economic and environmental nature.

	<p>Tip replacement in the calibration of piston-operated pipettes – economic and environmental implications</p> <p>https://doi.org/10.7795/550.20231106</p>	DKD-E 8-4	
		Edition:	10/2023
		Revision:	0
		Page:	12 / 13

7.1 Economic consequences

Increased demand for pipette tips leads to increased consumption of raw materials. This also applies to packaging, such as boxes for pipette tips.

An adequate supply of oil cannot be taken for granted.

The processing of raw materials also requires more energy.

The disposal of used pipette tips generates additional costs (again including energy costs).

And there are also additional costs for storage (construction costs or rental costs for storage facilities).

7.2 Environmental consequences

Plastic waste is a major problem for the oceans. This is why the European Union is planning to ban single-use plastic items (takeaway cups, straws, stirrers, cotton buds, etc.).

Pipette tips are a disposable product. In addition, the packaging (tip boxes) also accumulates as plastic waste.

Reducing the use of plastics is therefore an important goal for many industries.

Disposal by incineration in waste-to-energy plants is not an alternative. The result is an increase in CO₂ emissions.

8 Bibliography

- [1] ISO 8655-6: Piston-operated volumetric apparatus – Part 6: Gravimetric reference measurement procedure for the determination of volume (ISO 8655-6:2022, Corrected version 2022-06)
- [2] DKD-R 8-1: Calibration of piston-operated pipettes with air cushion, Version 12/2011, Deutscher Kalibrierdienst (DKD), Braunschweig



Published by:

Physikalisch-Technische Bundesanstalt
Deutscher Kalibrierdienst
Bundesallee 100
38116 Braunschweig

www.dkd.eu
www.ptb.de