Calibration of micropipettes using the air displacement method

Final Report

EURAMET research Project no. 1608

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IPQ-DMET - Volume and Flow Laboratory

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Contents

1. Introduction

During the EURAMET TCF 2024 the volume subgroup discussed the possibility of having a study on the calibration of micropipettes using the air displacement method [1] in the frame of a research project to test a new device and exchange experiences with this new method. UNIPIX, the manufacturer of this new device - ATMOS volunteered to cooperate with EURAMET in this project. Since most of the laboratories do not have this method implemented in the laboratory it was decided to register this project as a cooperation in form of a research project.
The Volume and Flow Laboratory of Portuguese Institute for Quality (IPQ) - National Laboratory of Metrology (NMI), acting as the pilot laboratory supplied the micropipettes and tips and performed the initial and final measurements of the micropipettes.
Three micropipettes (transfer package) were tested at different volume capacities.
This document presents results of this research project. The comparison schedule and participants are described in table 1.

Table 1 – Participants

Laboratory	Country	Responsible	Period
IPQ-1	Portugal	Elsa Batista ebatista@ipq.pt	January 2024
GUM	Poland	Ewa Malejczyk ewa.malejczyk@gum.gov.pl	February 2024
PTB	Germany	Tobias Nickschick tobias.nickschick@ptb.de	March 2024
MIRS	Slovenia	Urška Turnšek urska.turnsek@gov.si	April 2024
FORCE	Denmark	Lise-Lotte Grue llg@forcetechnology.com	May 2024
TUBITAK UME	Turkey	Gökce S. Sariyerli gokce.sariyerli@tubitak.gov.tr	July 2024
IMBIH	Bosnia and Herzegovina	Ela Brkić ela.brkic@met.gov.ba	August 2024
UniPix Sàrl	Switzerland	Daniel Bertrand daniel.bertrand@hiqscreen.com	September 2024
ZMK	Germany	Olaf Schnelle-Werner schnelle_werner@hotmail.com	November 2024
IPQ-2	Portugal	Elsa Batista ebatista@ipq.pt	December 2024

2. The instruments

The chosen instruments were 3 variable volume pipettes (see figure 1 (Eppendorf P34557G 1000 μL), figure 2 (Eppendorf 1839034 10 μL) and figure 3 (Eppendorf 8 multichannel P56623G 100 μL)) provided by IPQ. It was imperative that the micropipettes be equipped with a removable plastic tip, in order to facilitate the aspiration of liquid. IPQ supplied these tips. The Atmos devices (figure 4 and figure 5), and software were (used for data acquisition) supplied by UNIPIX.

Figure 1 - Variable Micropipette of 1000 mL

Figure 2 – Variable Micropipette of 10 mL

Figure 3 – Multichannel variable Micropipette of 100 mL

Figure 4- Single ATMOS

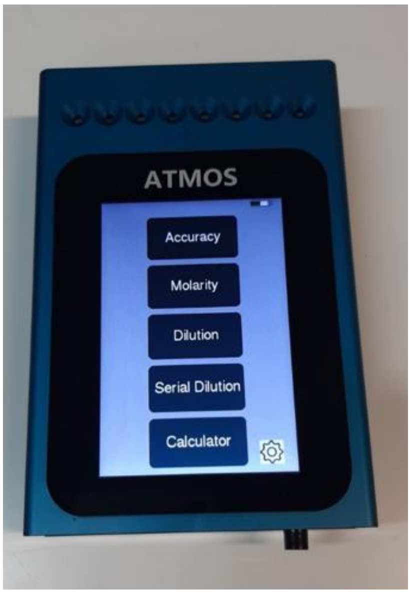

Figure 5- Multi ATMOS

3. Experimental tests

The 3 chosen instruments were tested at 100 %, 50 % and 10 % of the nominal volume, respectively.
Each test was performed with 10 replicates.
The pipette tip was inserted in the center of the tip port of the ATMOS. The pipette had to be hold vertically and the air inside the pipette had to be firmly pressed into the port.

A leak above 0.5 %/sec of each respective test volume (displayed by the calibration device) indicates a leaky pipette, or a leaky tip connection (or a bad insertion of the tip into the ATMOS port). These tests were to be discarded.

Throughout the 10 measurements, the tip was not to be replaced and maintained in the connecting port. As it is a calibration with air (dry), no liquid should ever be used.

The calibration values were directly taken from the ATMOS device at the ambient air temperature. No separate corrections for temperature were applied.

4. Calibration method

4.1 Method description

The concept of the air displacement method is to measure first the pressure increase (or decrease) caused by the piston displacement and introduce at a known timing the opening of an additional defined volume [1]. This strategy was initially described in relation to larger volumes. However, it has been demonstrated that it is applicable to volumes as small as a few microlitres, provided that the pressure resolution and stability requirements are met [1]. The principle of the reference volume is schematized in figure 6.

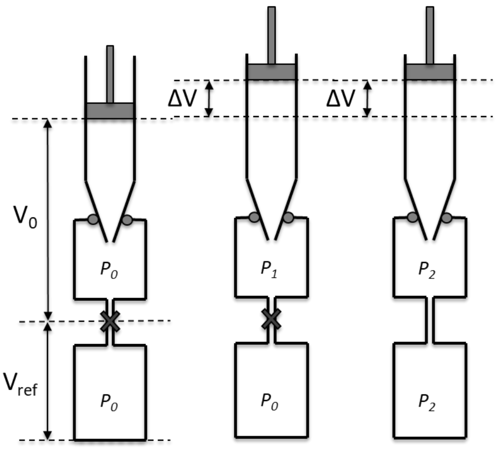

Figure 6: Schematic representation of the pressure measurement principle.

To begin, the tip insertion port and the valve of the reference volume (V_{ref}) are open, and the pressure is equilibrated with the atmosphere P_0. The valve of the V_{ref} volume is then closed and pressing the piston causes a reduction of the volume resulting in a pressure increase into the compartment V_0. Once the pressure is stabilized, its value is captured (P_1), the valve separating the V_{ref} volume is opened and the pressure P_2 is measured.

The difference in volume can be computed from the pressure differences in which P_0, P_1 and P_2 are the pressure values in hPa, V_{ref} is the reference volume in µL and ΔV corresponds to the volume displaced by the piston in µL.

$$\Delta V = V_{ref} / \left(\frac{P_1}{(P_1 - P_0)} - \frac{P_2}{(P_2 - P_0)} \right) \tag{1}$$

4.2 The measurement procedure

4.2.1 General

Press the main switch on the front of the instrument to power on. Press again to switch off.

- It is recommended to verify that the instrument battery is well charged or use the instrument with the external power supply connected via the USB cable.
- The instrument is powered by an internal Li-ion battery which allows continuous operating for more than 3 hours and several weeks with short daily testing sessions.
- After powering on, the instrument runs a self-test procedure for about 10 seconds.
- When not operated, the instrument automatically turns off after 5 minutes.

Select the pipette size to be tested.
After the pipette selection, or after measurements, the main panel is displayed.
From this panel, one can reach the following functions:

- The Insert Tip button will start the measurement procedure. Please insert the tip and be ready to actuate the piston BEFORE touching the button.
- The Pipette size button allows the selection of a new pipette model
- The Pipette ID button allows entering a numeric ID that will be stored with the measured data
- The Volume button allows the selection of sub-volumes of the pipette in the case of variable volume pipettes.
- The Settings panel can be reached by touching on the date & timeline.

4.2.2 Performing a calibration

- Insert the pipette tip in the center of the tip port of the instrument. Hold the pipette vertically and firmly press the tip into the port. When the tip is well inserted in the port, press the Start button without moving the pipette.
- Just after the “ready” message, quickly press on the pipette piston while firmly keeping the tip inserted in the port. The displacement of the piston has to be done in about 1 to 2 seconds during the BLUE phase of progress bar.
- Do not press the piston beyond the first stop.
- The progress bar at the bottom indicates that the instrument is measuring. The pipette and the piston should not move when the progress bar is RED.
- When the release display appears, move the piston up again without removing the pipette from the port.
- The device shows the measured volume and the leak rate (expressed in volume loss per second).
- Select “next” and start next measurement without removing the pipette tip from the port.
- The END button will stop the series and display statistics.

5. Evaluation of the measurement results

5.1 Reference value

It was decided to use the same statistical analysis as in any supplementary comparison even though the main focus was to gain experience with this dry calibration method and ATMOS device. Therefore to determine the reference value the formula of the weighted mean is used. The weighing factors are the inverses of the squares of the associated standard uncertainties [2]:

$$y = \frac{x_1 / u^2(x_1) + ... + x_n / u^2(x_n)}{1/u^2(x_1) + ... + 1/u^2(x_n)} \quad . \qquad (2)$$

To determine the standard uncertainty u(y) associated with y the following expression is used:

$$u(y) = \sqrt{\frac{1}{1/u^2(x_1) + ... + 1/u^2(x_n)}} \quad . \qquad (3)$$

5.2 Consistency determination

To identify an overall consistency of the results, a chi-square test is applied to all *n* calibration results.

$$\chi^2_{obs} = \frac{(x_1 - y)^2}{u^2(x_1)} + ... + \frac{(x_n - y)^2}{u^2(x_n)} \qquad (4)$$

where the degrees of freedom are: $\nu = n - 1$

The consistency check is regarded as failed if: $\Pr\{\chi^2(\nu) > \chi^2_{obs}\} < 0{,}05$. The function *CHIINV(0,05; n-1)* in MS Excel was used. The consistency check was failing if *CHIINV(0,05; n-1) <* χ^2_{obs}.

If the consistency check did not fail, then *y* was accepted as the Reference Value (RV) x_{ref} and $U(x_{ref})$ was accepted as the expanded uncertainty of the RV.

If the consistency check failed then the laboratory with the highest value of $\frac{(x_i - y)^2}{u^2(x_i)}$ is excluded from the next round of evaluation and the new reference value, reference standard uncertainty and chi-squared value is calculated again without the excluded laboratory.

The E_n value was also calculated. This value is defined as [2,3]:

$$E_{n_{lab-i}} = \frac{\varepsilon_{lab-i} - \varepsilon_{RV}}{\sqrt{U^2(\varepsilon_{lab-i}) - U^2(\varepsilon_{RV})}} \qquad (5)$$

where ε_{lab-i} is the error of lab-i for a certain point, ε_{RV} is the comparison reference value (RV) for the error and $U(\varepsilon_{lab-i})$ and $U(\varepsilon_{RV})$ and the expanded uncertainties (*k*=2) of those values.

With the value of E_n one can conclude that:

- The results of the laboratory for a certain point are consistent (passed) if $E_n \leq 1$

– The results of the laboratory for a certain point are inconsistent (failed) if $E_n > 1$

IPQ performed two calibrations, one at the beginning and another at the end of the to access the stability of the artefacts.
The first result of IPQ was considered for the determination of reference value, along with its value of uncertainty.

In the results analysis the maximum permissible systematic errors of the micropipettes were used. These values were taken from ISO 8655-2 [4]:

- 8 μL for all the points of the 1000 μL micropipette
- 0,1 μL for all the points of the 10 μL micropipette
- 1,6 μL for all the points of the 100 μL multichannel micropipette

6. Measurement results

6.1. Determination of the stability of the artefacts

In order to determine the reference value and access the stability of the instrument, two measurements were performed by IPQ - one at the beginning and another at the end of the comparison for the 3 instruments.

Table 2 – Stability of the transfer standards

	IPQ1		IPQ2		
1000 μL Micropipette	**Volume (μL)**	**Uncertainty (μL)**	**Volume (μL)**	**Uncertainty (μL)**	**ΔV(μL)**
1000	999,1	2,5	1000,3	2,4	1,2
500	499,9	1,5	500,23	1,2	0,33
100	102,28	0,46	101,93	0,34	-0,35
10 μL Micropipette	**Volume (μL)**	**Uncertainty (μL)**	**Volume (μL)**	**Uncertainty (μL)**	**ΔV(μL)**
10	10,70	0,041	10,71	0,048	0,01
5	5,62	0,044	5,67	0,066	0,05
1	1,60	0,043	1,63	0,055	0,03
100 μL Multi-Micropipette Channel 1	**Volume (μL)**	**Uncertainty (μL)**	**Volume (μL)**	**Uncertainty (μL)**	**ΔV(μL)**
100	99,79	0,31	100,15	0,39	0,36
50	49,55	0,32	49,60	0,23	0,15
10	9,89	0,21	10,08	0,13	0,19

The resulting variations of IPQ are smaller than the declared uncertainty of the first test and therefore it is assumed that all instruments were stable during the comparison. This was not observed in all laboratories, especially in case of the 1000 μL pipette.

6.2. Volume results with reference value

6.2.1 1000 µL Micropipette

Due to a large leakage value, FORCE and PTB results were not considered in this report for the 1000 µL micropipette.

Table 3 – Volume measurement results – Micropipette 1000 µL

Laboratory	Volume (µL)	Uncertainty (µL)
IPQ	999,1	2,5
GUM	1004,4	2,4
MIRS	1001,0	2,1
TUBITAK	999,8	2,5
IMBIH	986,3	2,7
UNIPIX	995,0	2,9
ZMK	991,1	2,6
IPQ final	1000,3	2,4
Vref	997,36	0,94

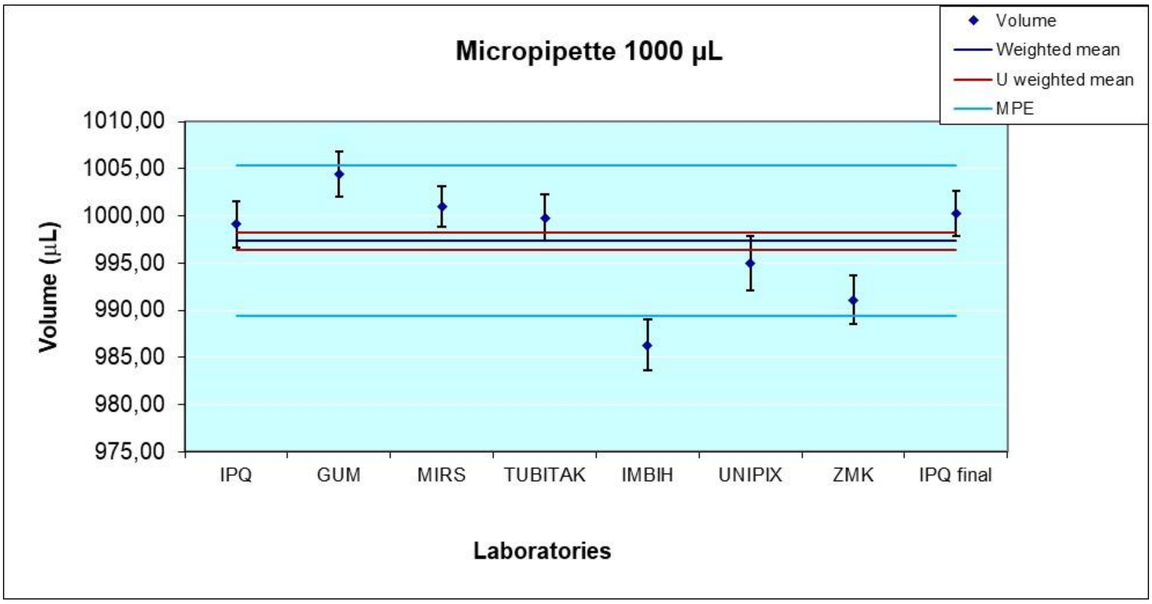

Figure 7 – Volume results with reference value – micropipette 1000 µL

From figure 7 it can be verified that there are 3 results consistent with the reference value determined by the weighted mean, but it is not possible to decide the largest consistent subset and perform a chi-square test. One result is outside the ISO standard [4] specification for the systematic error of pipettes (MPE).

Table 4 – Volume measurement results – Micropipette 500 μL

Laboratory	Volume (μL)	Uncertainty (μL)
IPQ	499,9	1,5
GUM	501,7	1,2
MIRS	501,8	1,8
TUBITAK	498,8	1,4
IMBIH	495,9	1,2
UNIPIX	497,4	1,4
ZMK	489,3	2,0
IPQ final	500,2	1,2
Vref	498,32	0,54

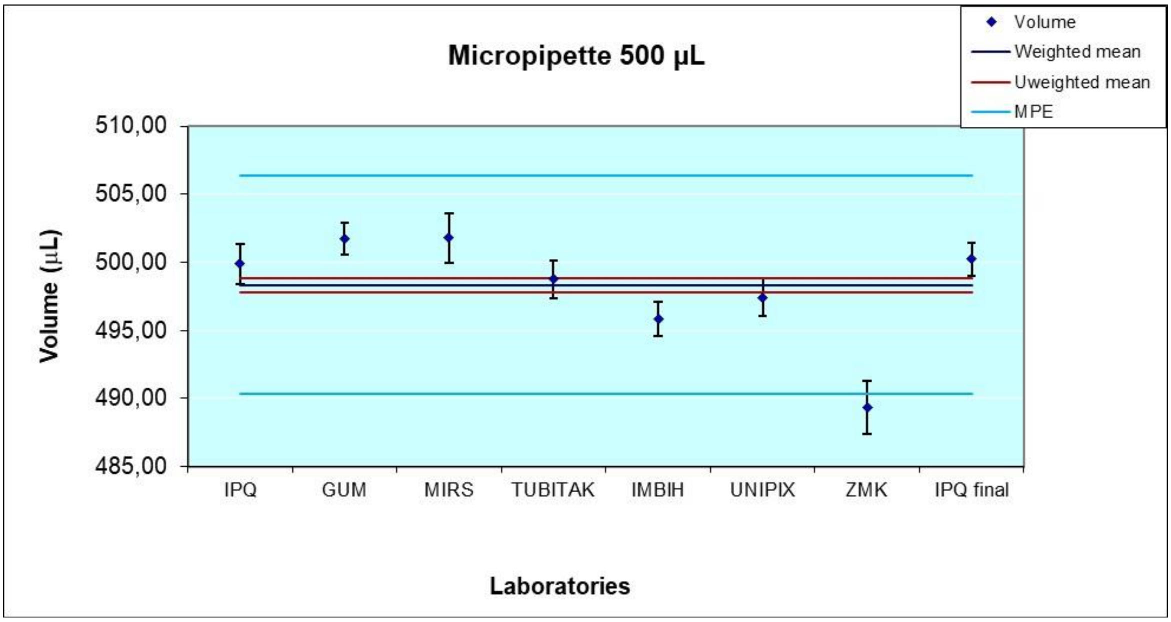

Figure 8 – Volume results with reference value – micropipette 500 μL

From figure 8 it can be verified that there are 3 results consistent with the reference value determined by the weighted mean, but again it is not possible to decide the largest consistent subset and perform a chi-square test. All results are within ISO standard [4] specification for the systematic error of pipettes (MPE).

Table 5 – Volume measurement results – Micropipette 100 μL

Laboratory	Volume (μL)	Uncertainty (μL)
IPQ	102,28	0,46
GUM	102,33	0,50
MIRS	102,84	0,74
TUBITAK	102,53	0,28
IMBIH	101,47	0,57
UNIPIX	100,62	0,60
ZMK	99,75	1,76
IPQ final	102,28	0,46
Vref	102,16	0,18

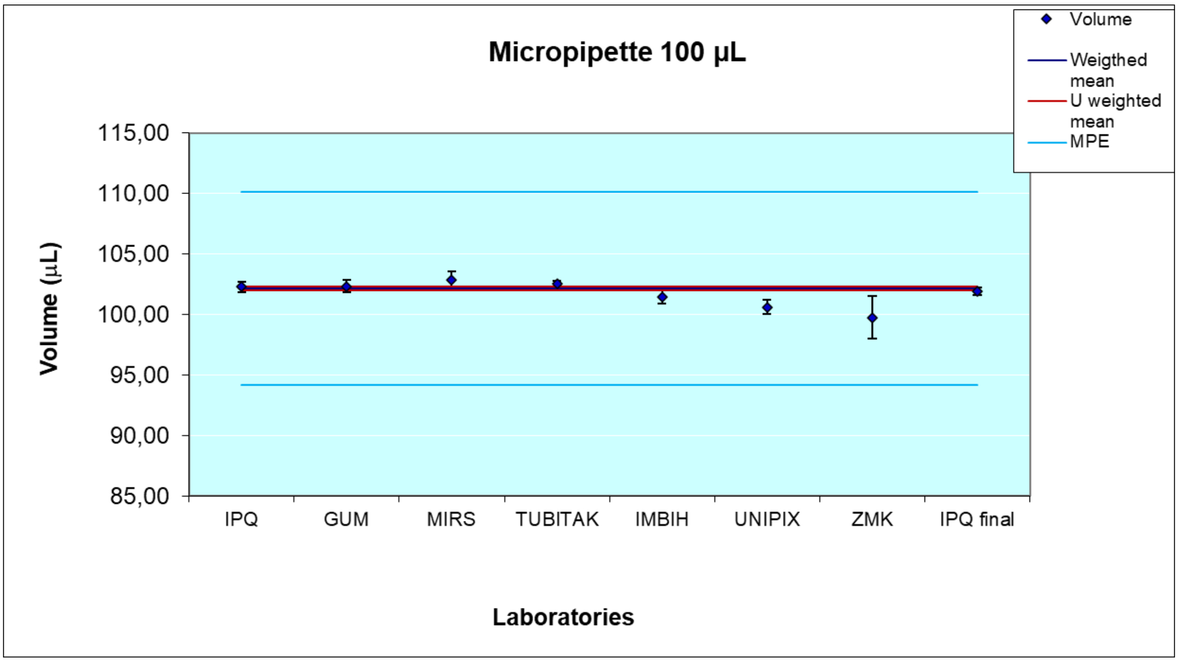

Figure 9 – Volume results with reference value – micropipette 100 μL

From figure 9 it can be verified that there are 5 results consistent with the reference value determined by the weighted mean. All results are within the ISO standard [4] specification for the systematic error of pipettes (MPE).

6.2.2 10 μL Micropipette

Table 6 – Volume measurement results – Micropipette 10 μL

Laboratory	Volume (μL)	Uncertainty (μL)
IPQ-1	10,70	0,041
GUM	10,85	0,052
PTB	10,77	0,250
MIRS	10,70	0,054
FORCE	10,43	0,054
TUBITAK UME	10,67	0,061
IMBIH	10,47	0,042
UNIPIX	10,39	0,080
ZMK	10,54	0,045
IPQ-2	10,70	0,041
Vref	10,604	0,018

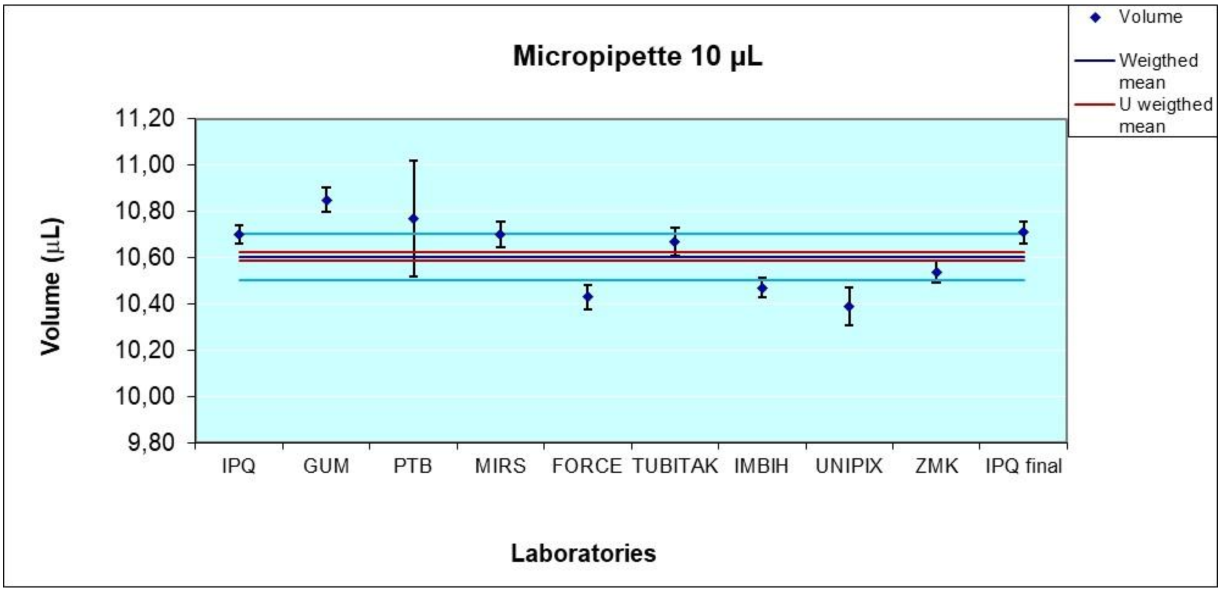

Figure 10 – Volume results with reference value – micropipette 10 μL

From figure 10 it can be verified that there are only 3 results consistent with the reference value determined by the weighted mean, but it is not possible to decide the largest consistent subset and perform a chi-square test. Three results are outside the ISO standard [4] specification for the systematic error of pipettes (MPE).

Table 7 – Volume measurement results – Micropipette 5 μL

Laboratory	Volume (μL)	Uncertainty (μL)
IPQ-1	5,62	0,044
GUM	5,65	0,031
PTB	5,56	0,090
MIRS	5,51	0,079
FORCE	5,56	0,055
TUBITAK UME	5,67	0,064
IMBIH	5,44	0,047
UNIPIX	5,42	0,060
ZMK	5,55	0,036
IPQ-2	5,62	0,044
Vref	5,571	0,016

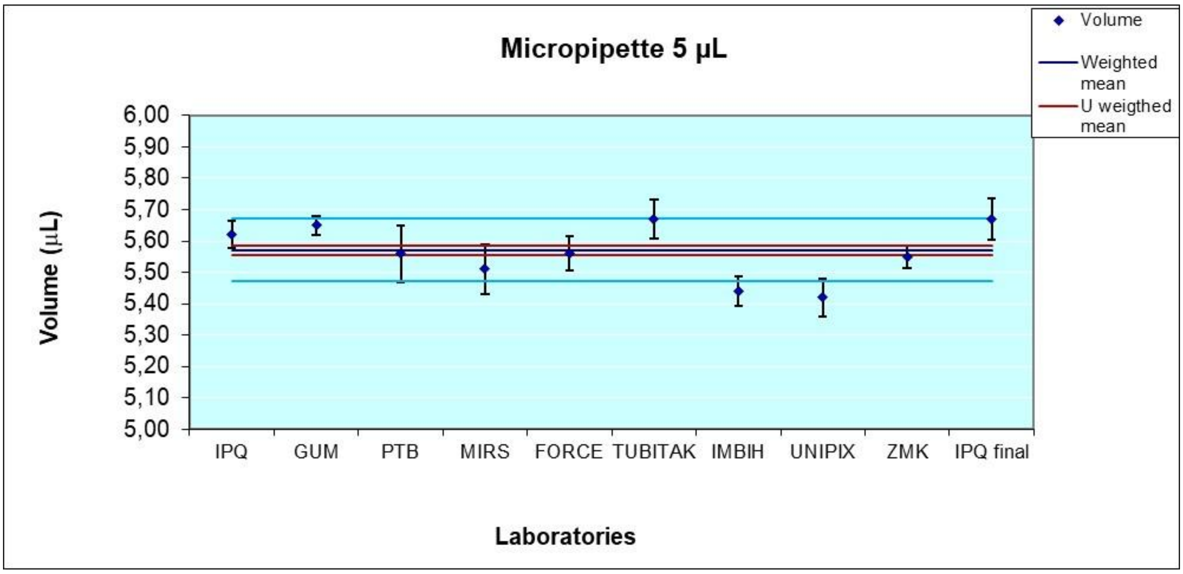

Figure 11 – Volume results with reference value – micropipette 5 μL

From figure 11 it can be verified that there are 5 results consistent with the reference value determined by the weighted mean. All the results are inside the manufacturer specification for the systematic error (MPE).

Table 8 – Volume measurement results – Micropipette 1 μL

Laboratory	Volume (μL)	Uncertainty (μL)
IPQ-1	1,60	0,043
GUM	1,65	0,020
PTB	1,49	0,050
MIRS	1,48	0,062
FORCE	1,47	0,022
TUBITAK UME	1,64	0,047
IMBIH	1,40	0,036
UNIPIX	1,45	0,110
ZMK	1,46	0,026
IPQ-2	1,60	0,043
Vref	1,533	0,011

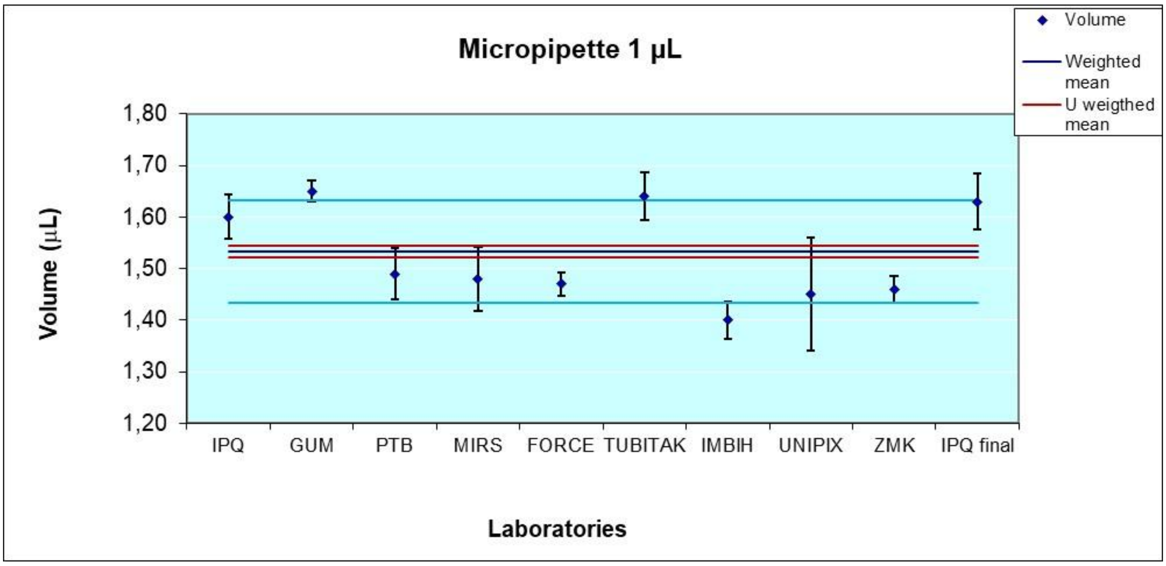

Figure 12 – Volume results with reference value – micropipette 1 μL

From figure 12 it can be verified that there are 3 results consistent with the reference value determined by the weighted mean. All results are inside the manufacturer specification for the systematic error (MPE).

6.2.3 100 μL Multichannel Micropipette

The results for channel 1 are presented in detail. The results for the other channels are represented in figures 14, 16 and 18. The uncertainty values are similar for all channels.

Table 9 – Volume measurement results – Micropipette 100 μL -channel 1

Laboratory	Volume (μL)	Uncertainty (μL)
IPQ-1	99,79	0,31
GUM	99,53	0,26
PTB	100,52	0,35
MIRS	99,75	0,66
FORCE	98,56	0,34
TUBITAK UME	101,16	0,45
IMBIH	99,30	0,26
UNIPIX	98,46	0,68
ZMK	99,80	0,51
IPQ-2	100,15	0,39
Vref	99,62	0,12

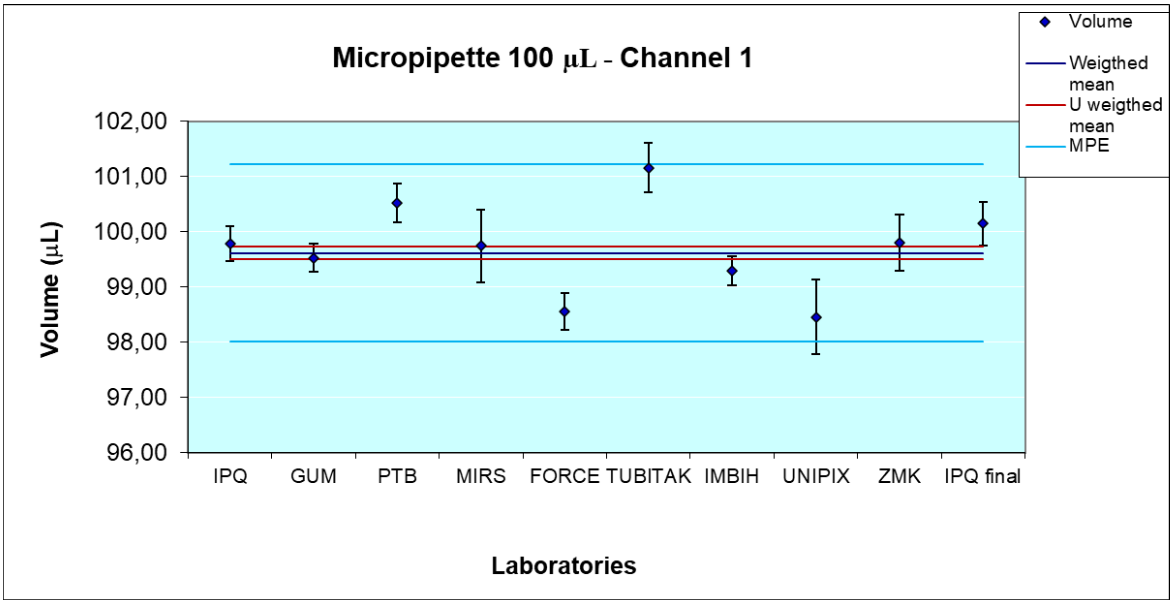

Figure 13 – Volume results with reference value – micropipette 100 μL

From figure 13 it can be verified that there are 5 results consistent with the reference value determined by the weighted mean. All results are within the manufacturer specification for the systematic error (MPE).

The values for all the channels and all the participants are presented in figure 14.

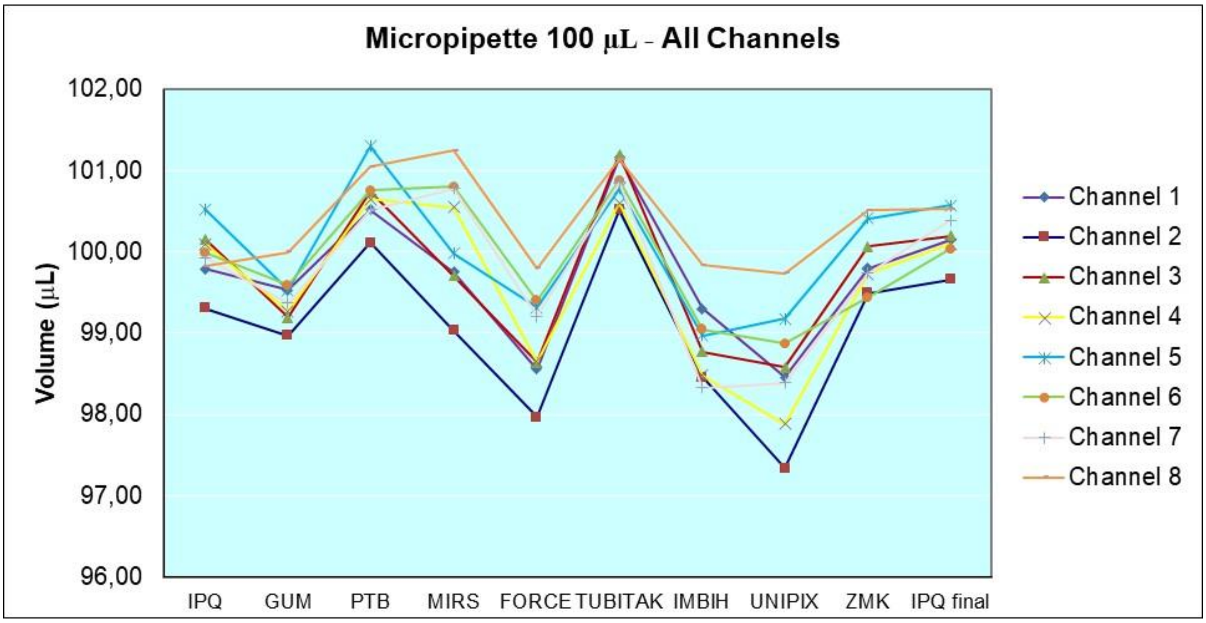

Figure 14 – Volume results for all channels – micropipette 100 µL

Table 10 – Volume measurement results – Micropipette 50 µL – channel 1

Laboratory	Volume (µL)	Uncertainty (µL)
IPQ-1	49,55	0,32
GUM	49,23	0,26
PTB	50,82	0,18
MIRS	49,97	0,63
FORCE	49,84	0,35
TUBITAK UME	51,54	0,24
IMBIH	49,47	0,19
UNIPIX	49,30	0,51
ZMK	49,75	0,36
IPQ-2	49,60	0,23
Vref	50,13	0,090

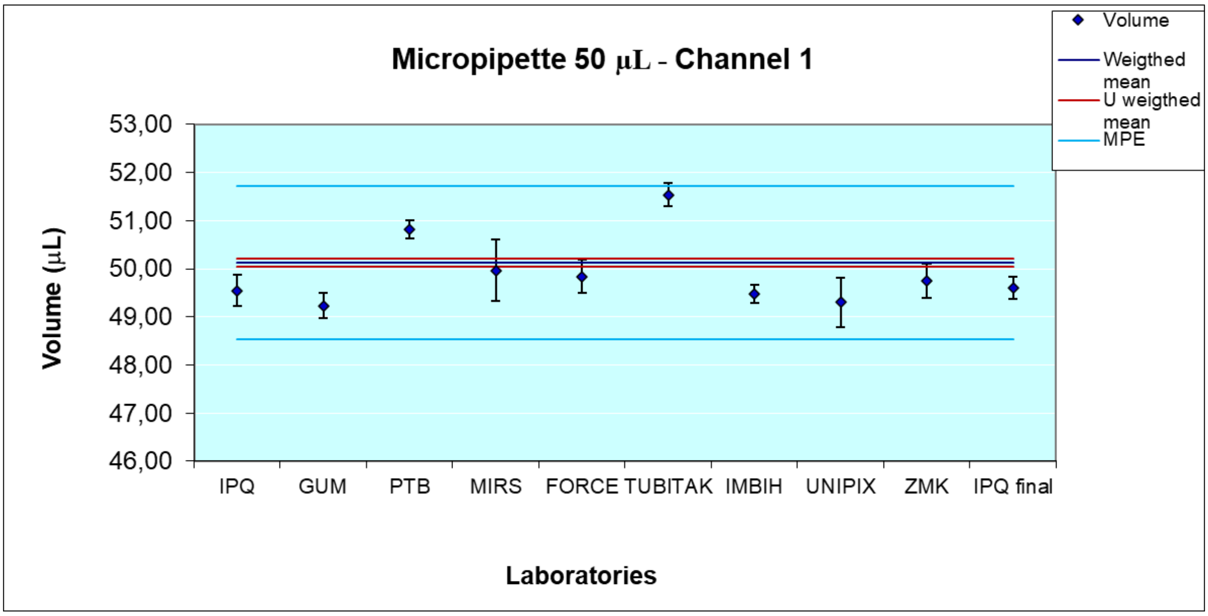

Figure 15 – Volume results with reference value – micropipette 50 µL

From figure 15 it can be verified that there are 3 results consistent with the reference value determined by the weighted mean, but it is not possible to decide the largest consistent subset and perform a chi-square test. One result is outside the manufacturer specification for the systematic error (MPE).

The values for all the channels and all the participants are presented in figure 16.

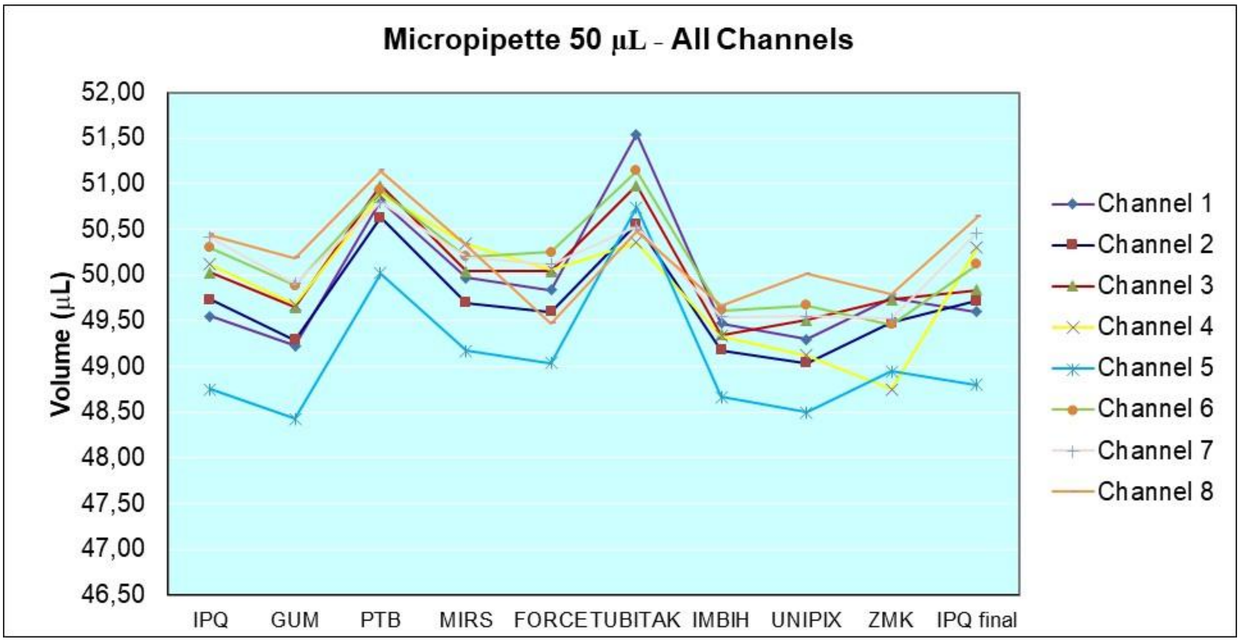

Figure 16 – Volume results for all channels – micropipette 50 µL

Table 11 – Volume measurement results – Micropipette 10 μL – channel 1

Laboratory	Volume (μL)	Uncertainty (μL)
IPQ-1	9,89	0,21
GUM	9,87	0,33
PTB	10,64	0,22
MIRS	10,07	0,37
FORCE	10,24	0,13
TUBITAK UME	10,68	0,12
IMBIH	10,34	0,13
UNIPIX	9,50	0,14
ZMK	10,19	0,22
IPQ-2	10,08	0,13
Vref	10,01	0,13

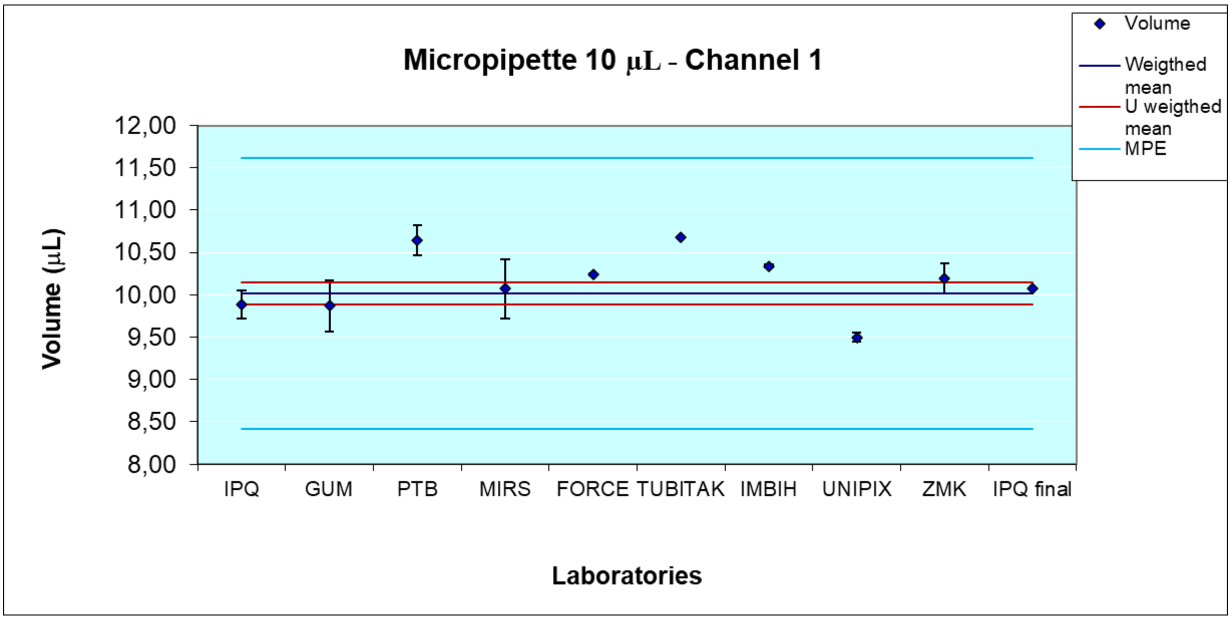

Figure 17 – Volume results with reference value – micropipette 10 μL

From figure 17 it can be verified that there are 4 results consistent with the reference value determined by the weighted mean. All results are inside the manufacturer specification for the systematic error (MPE).

The values for all the channels and all the participants are presented in figure 18.

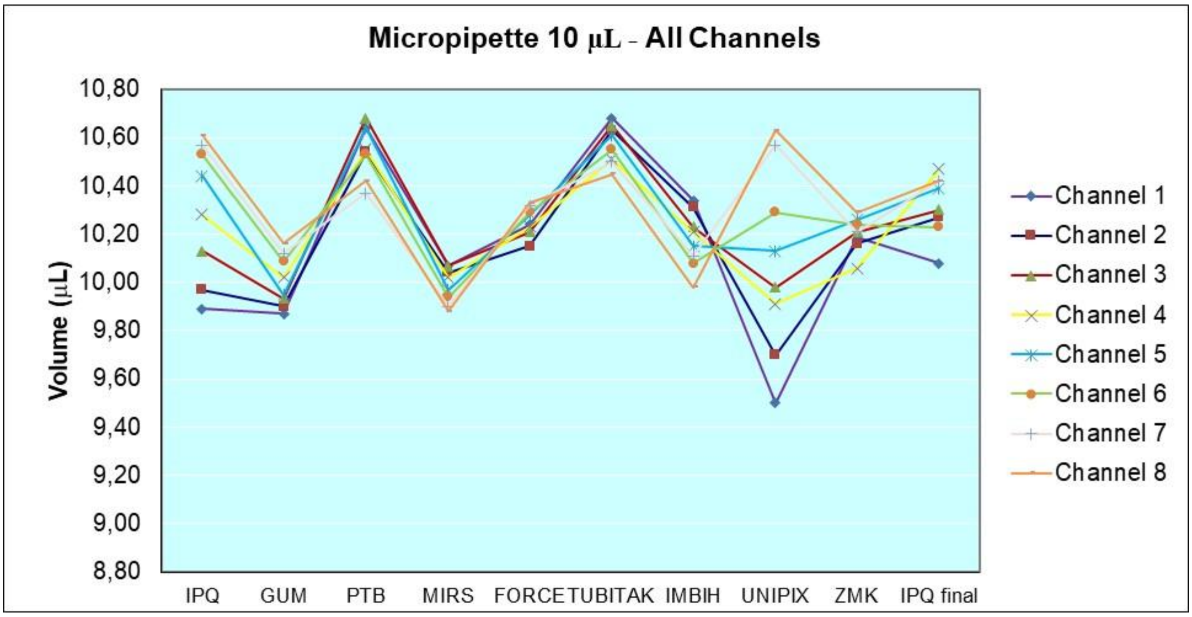

Figure 18 – Volume results for all channels – micropipette 10 µL

6.2.4 Relative degree of equivalence for the single channel micropipettes

In order to verify the relative variation of the laboratories for the single channel micropipettes regarding the reference value, the following figure 19 was prepared that included relative results from all tested points.

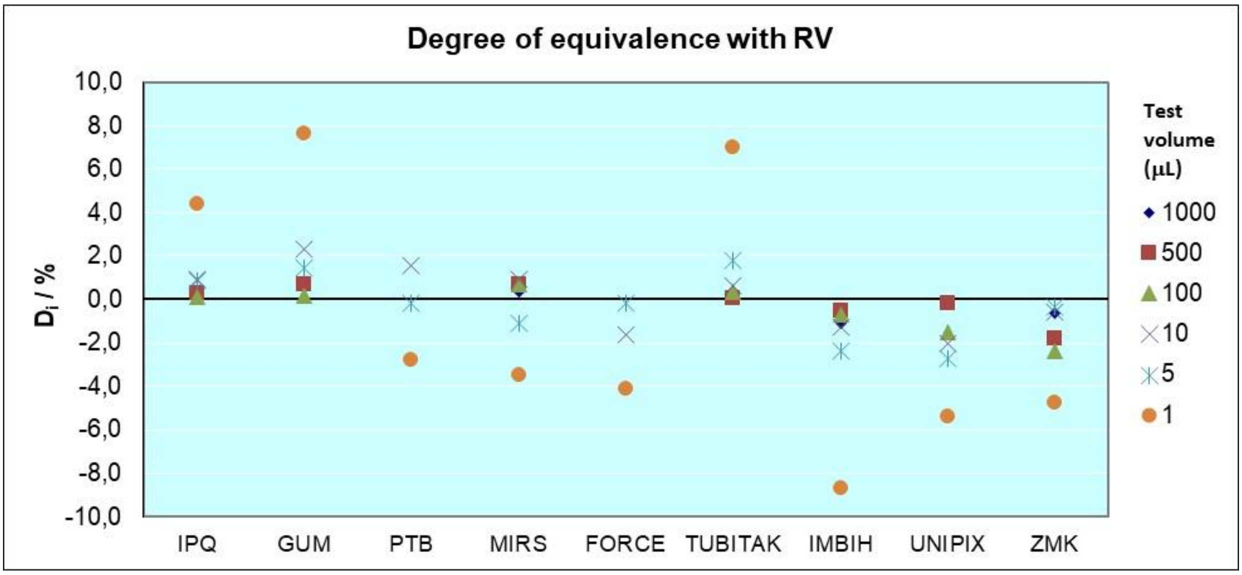

Figure 19 – Relative degree of equivalence single channel micropipettes

It has been demonstrated that there is a direct correlation between the volume and the relative variation, with a higher volume corresponding to a higher relative variation.

7. Uncertainty calculation

The uncertainty of pipette calibration for all participants was estimated following the Guide to the Expression of Uncertainty in Measurement (GUM) [5].
The main contributions for the standard uncertainty were agreed between the laboratories considering that this is a new testing method. The components are the following: the repeatability of the measurements, ATMOS resolution, ATMOS accuracy and the reproducibility of measurements.

Table 12 - Uncertainty components in the calibration of a micropipette using the air displacement method

Source / Symbol	Standard uncertainty component	Explanation	Evaluation type	Distribution
Repeatability of measurements	urep	Mean standard deviation	A	Normal
Accuracy of ATMOS declared by manufacturer	uacc	Value declared by manufacturer (0,1 %)	B	Rectangular
Resolution of ATMOS	ures	Resolution of Atmos	B	Rectangular
Reproducibility of measurements	urepr	EURAMET guide cg19 (0,1 %)	B	Rectangular

Due to all participants using the same values for the uacc, ures and urepr, the main contributing factor of each laboratory's uncertainty was their respective results from repeatability. Also, it was identified that some components of the method could be missing, but it was not possible to identify them yet.

8. Comparison with gravimetric method

Several laboratories performed the calibration of the micropipettes using both displacement method [1] and gravimetric method [6]. Some variations were observed by all participants (figure 20 and 21), in general the values for the displacement method are higher than the gravimetric method. The relative offset was significantly higher at lower volumes.

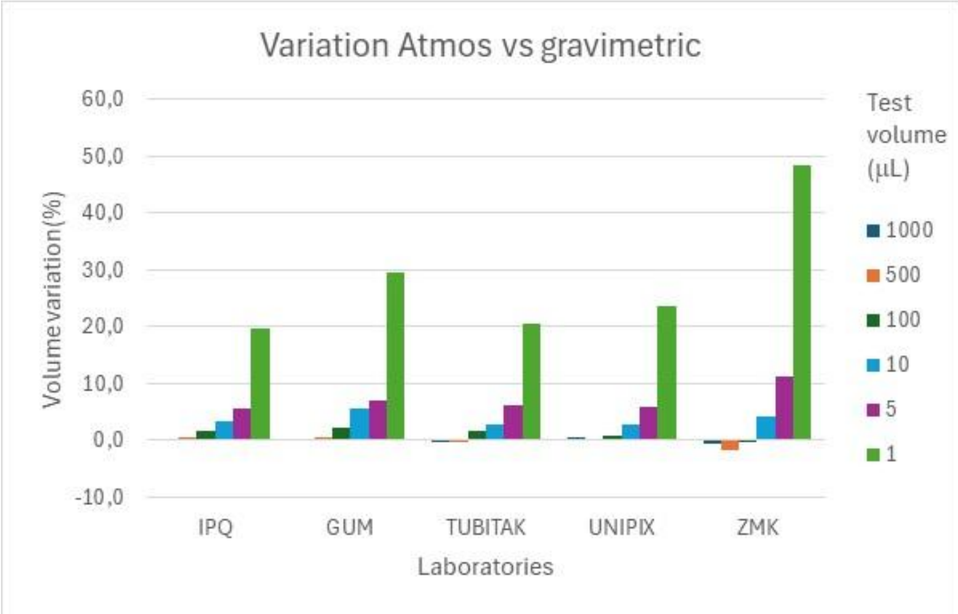

Figure 20 – Relative degree of equivalence between displacement method and gravimetric method

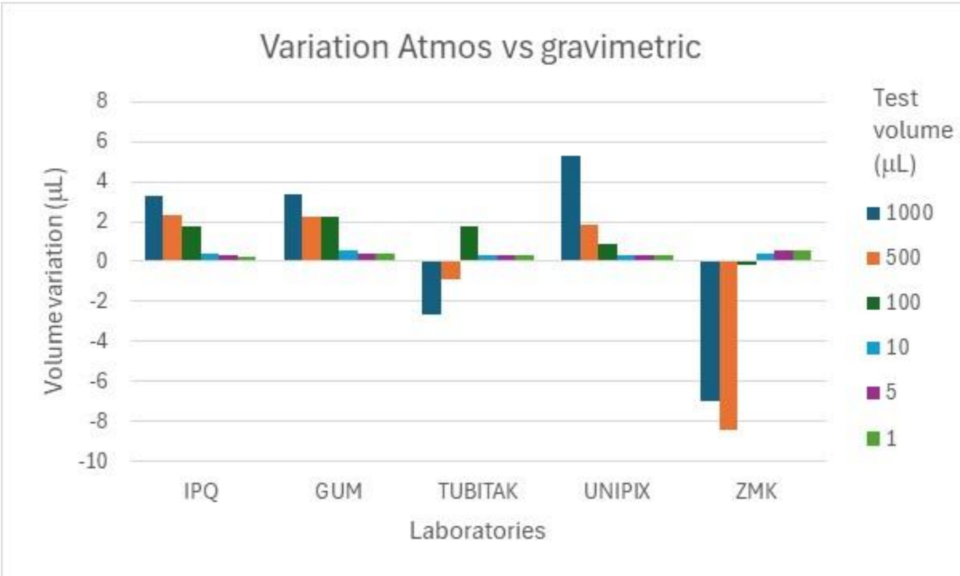

Figure 21 – Absolute variation between displacement method and gravimetric method

9. Conclusions

A new micropipette test method was developed by UNIPIX that uses the concept of air displacement inside the pipette caused by the movement of the piston. In order to verify if the method is robust and reproducible, a research project was performed by several Europeans NMIs, an accredited laboratory and the manufacturer of the ATMOS device used in this study. Three micropipettes of different types and volumes were tested using two different ATMOS instruments in total. Due to leakage problems, 2 laboratories' results were not considered in the evaluation of the 1000 μL micropipette.

From statistical analysis results were in general, for all pipettes, 50 % consistent with the reference value, but it was not possible to identify the largest consistent subset of results in order to perform the statistic evaluation according to the Cox method [2]. An analysis revealed a certain degree of variation in the expanded uncertainty declared by the participants. This variation was primarily attributable to the repeatability of the measurements. In principle, the uncertainty of the method is underestimated by all participants due to some existing uncertainty components not identified yet.

For the majority of the participants, this was a first contact with this new method so it was expected that some variations in the results and the uncertainty would arise due to limited or non-existent experience of the technicians in handling of the ATMOS instrument, mainly: the velocity of compression, the tightness of the tip when pressed, how steady the instrument is kept during each repetition and at what point during each step (marked by red or blue bars) the pipette is pressed or released, the verticality of the pipette position, among others. These variations are more significant at lower volumes where a small pressure variation could lead to large volume variability. Also, some variations were observed in all participants when comparing the displacement method with the gravimetric method, in general the values for the displacement method are higher than the gravimetric method. The offset was higher at lower volumes.

The laboratories have made some comments on their experience with this method and some remarks can be found in Annex 1.
Finally, when the results are compared with the manufacturer specification of the pipette they are to 90 % in agreement., This indicates that the air displacement method could be very useful for a simple end users conformity evaluation of their pipettes.

10. References

1. D. Bonzon et al 2019 Meas. Sci. Technol. 30 105003
2. M.G. Cox, The evaluation of key comparison data, Metrologia, 2002, Vol. 39, 589-59
3. ISO 13528:2005 - Statistical methods for used in proficiency testing by interlaboratory comparisons
4. ISO 8655-2:2022 – Piston-operated volumetric apparatus — Part 2: Pipettes
5. JCGM 100:2008 -Guide to the expression of uncertainty in measurement (GUM), BIPM, IEC, IFCC, ISO, IUPAC, IUPAP, OIML; Geneva, 1995
6. ISO 8655-6:2022 – Piston-operated volumetric apparatus — Part 6: Gravimetric reference measurement procedure for the determination of volume

Annex 1 – Comments made by participants regarding this new displacement method

IMBIH

- The calibration data for pressure sensor(s) and referent volume (Vref) should be available to be able to get the true values and to calculate measurement uncertainty.

- The device measures the temperature but we don't know what and where and this temperature is usually 2 or 3 degrees bigger then environment. Also, the volume is calculated from two pressures, without temperatures (!?)

- In the downloaded files for both devices, the resulting volume (dV) can not be reproduced from the data in the file (Vref, P1, P2), i.e. the calculated values differs from displayed. Probably this is the problem with rounding but …

- For the single channel device, the leak is shown in percentage, while in the downloaded file, according to the formula, it is in volume units.

- In the downloaded file for a multichannel micropipette, there is no data for leak, i.e. the value of leak is only available on display during measurement.

- The formula for calculating the leak, which is applied for a single-channel micropipette, doesn't work for a multi-channel micropipette, i.e. the calculated values doesn't agree with values on the display of device.

PTB

- As a general notice: the test measurements with the largest pipette (1000 µl) resulted in higher leakage values (~2.0 %) – above the required < 0.5%. It is currently uncertain whether this is related to the pipette, the tip or the ATMOS' port openings and their respective sealing. No change in approach (slightly different amounts of pressure applied downwards, keeping the pipette absolutely still) could eliminate this effect.
- Attention should be paid when using the multi-channel pipette to not twist the bottom part and mixing up the order: Channel 1 needs to be left, channel 8 needs to be on the right side to not mix up the respective order of pipette tip and channel recorded.
- The measurement is very susceptible to even the slightest movement during the actual measurement. Even breathing seems to affect the measurement negatively – not related to "breathing air onto the ATMOS" but the slight changes in angle when the pipette tip is inserted. Not breathing while the pressure measurement takes place seems to result in significantly more homogeneous repetition values.

- We additionally recorded the time stamp for the measurement (which was not explicitly required by the guide) but we concluded the time to be crucial for the correlation between manually written values and the data set stored within the device.
- Two different people carried out the measurements: an experienced user (Christian Franke, PTB) and a novice (Tobias Nickschick, PTB) to potentially record differences when handling the test objects. Both data sets will be delivered just to have more data if needed.
- The total time spent on characterizing the 3 pipettes at 3 specific calibration points by 2 different observers took about 3 hours to get a feeling for the pipettes – additional heating of the pipettes by body heat is expected from the first tests on March 6th.

FORCE

- FORCE Technology did not make a "self test".
- To include pressure in the uncertainty we need some information on the equipment
- Calibrating the 1000 µl pipette /tips, we had problems with leak

ZMK

- In accordance with the Technical Protocol, it was also possible to carry out the gravimetric calibration according to ISO 8655.
- We have done this and also forward the measuring values to the pilot laboratory.
- The comparison between the air displacement method and the gravimetric method showed relatively good agreement up to 100 µl.
- However, a larger offset was observed for volumes > 100 µl.

IPQ

- The device ATMOS was easy to use but required some training time in order to have handling stability and results consistency.
- In the multichannel pipettes its difficult not to have leakage in the external channels, 1 and 8 because the pressure in the fit its done in the central channels.
- The smaller the volume the more difficult is to have consistency.
- We believe that this device will be very useful for pipettes intermediate verification by the end user.