On the microvolume measurement from 0.1 µL up to 100 µL using a micropipette

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Abstract. Measuring a microvolume with a micropipette allows for great time optimization and does not require as much skill from the user, when compared to the same measurement using a microsyringe. Even with all these advantages, there are details in measurement that cannot be overlooked. The main objective of this study is to characterize the behavior of micropipettes during the measurement of microvolumes in the range between 0.1 and 1 µL by the gravimetric method, with metrological traceability, and present the results in the range up to 100 µL. The ISO 8655-6:2022 standard specifies a measurement procedure by the gravimetric method for determining the volume of piston-operated volumetric instruments. For measurement below 1 µL, some influences are very significant and must be considered at the time of measurement. Due to the great difficulty in aspirating volumes of 0.1 µL, greater attention must be applied in order to avoid values that are very different from what is expected. Another important point is that the value of 100 µL value is the point where the balance resolution, when compared to balances with 0.01 mg and 0.001 mg resolution, does not impact the expanded uncertainty of the measurement. From this point on, other sources of uncertainty assume greater influence.

1. Introduction

Metrology is a science that involves people's daily lives. Everything one does, every plan, everything involves measurement. From a step to the flow of air we breathe, it is possible to quantify, dose or measure several quantities interacting at the same time.

From the beginning of civilizations to the present day, the advancement of technology depends a lot on increasingly accurate knowledge, improvement and creation of new measurement methods. The 9th edition of the International System of Units (SI) defined fixed numerical values of a set of seven constants from which the definitions of the seven base units of the SI would be deduced. This interaction between metrology and different sectors of research and industry makes it possible to understand things better and, consequently, to develop new products and services.

Volume is a unit derived from SI base units and is expressed by m^3. However, it is also possible to express the measurement of volume in the liter unit and its submultiples (deciliter, centiliter, milliliter, microliter, among others). Its measurement is closely aligned with people's routine. We measure the volume of water we must drink during the day; we know the volume of blood in our body and even the amount of medicine we must take depending on our weight. With greater accuracy and on a smaller scale, microvolume measurement is very important and is linked to new technologies such as biotechnology and nanotechnology.

The main instruments involved in microvolume measurement are micropipettes and microsyringes. Micropipettes are instruments most used in laboratories and work by activating a piston that can be mechanical or electronic. In most cases, the aspirated liquid has no contact with the micropipette, as it is stored in a disposable tip, which helps to avoid contamination of samples and its greater versatility. Micropipettes can be single-channel or multi-channel, where they are usually 8 or 12 channels. For laboratory activities where the user needs to make repeated measurements, the multichannel micropipette is widely used and optimizes the work.

Microsyringes are more used in chromatography or for microvolume measurements that require greater accuracy. It is a graduated instrument, and its operation is by a plunger that dispenses the previously aspirated liquid. In this case, the liquid meets the needle and the internal capillary of the microsyringe, in addition, it is a slower measurement and requires special care to ensure good results. In this work, all measurements are made using a mechanical single-channel micropipette.

Figure 1 (a): **Single-channel and multi-channel micropipette**

Figure 1 (b): **Microsyringe**

The main objective of this study is to characterize the behavior of micropipettes during the measurement of microvolumes in the range between 0.1 and 1 μL by the gravimetric method, with metrological traceability, and present the results in the range up to 100 μL.

2. State of Art on micropipettes

Until the middle of the 20th century, the glass pipette was the most accurate laboratory glassware, but for low volumes the repeatability achieved compromised the tests. Another factor that compromised the measurements was the risk of contamination when sucking the liquid with the mouth.

In the 1950s the micropipette was developed by reconstructing a syringe. A spring was added to the piston which, when finding a limiter at the top, defined the volume to be measured. The needle syringe was replaced by a plastic tip that was attached to a hermetic plastic tube where, when pipetting, an air pocket was formed that separated the liquid from the syringe piston. Now, all contact would only happen with the plastic tip. “Micropipettes are instruments used to measure very small amounts of liquid, in the order of a microliter” (Batista, et al. 2016).

The techniques employed in the construction of the first micropipettes are like those of a currently developed model. With the evolution of technology, the new features that were coupled served to improve repeatability and ergonomics, increase the range of use, electronic resources and the use of multichannel. Currently, the measurement range of micropipettes is very wide, and it is possible to find equipment from 0.1 μL to 50 mL.

Measuring a microvolume with a micropipette allows for great time optimization and does not require as much skill from the user, when compared to the same measurement using a microsyringe. Even with all these advantages, there are details in measurement that cannot be overlooked. The ISO

8655-6:2022 standard specifies a measurement procedure by the gravimetric method for determining the volume of piston-operated volumetric instruments. However, according to Batista et al. 2019, there are several experimental parameters that can influence micropipette calibration. These parameters can lead to inconsistent and inaccurate results and are not explained in detail in the ISO 8655-6 standard, such as: time to aspirate and dispense the volume, variation in the angle of aspirating and dispensing the liquid, tip immersion depth, the type of tip used and the altitude.

Figure 2: **Micropipette**

For measurement below 1 µL these influences are also significant and must be considered at the time of measurement. Due to the great difficulty in aspirating volumes of 0.1 µL, greater attention must be applied to avoid values that are very different from what is expected.

3. Gravimetric Method

Volume measurement by the gravimetric method is done by measuring the mass of the liquid on a balance under specific conditions. This liquid, which the mass will be measured, needs to have a known density value, since the volume is a ratio between the density and the mass, according to the equation (1).

$$V = \frac{\rho_{water}}{m_{water}} \qquad (1)$$

Where ρ_{water} is the density of water, and m_{water} is the mass of water measured on the balance.

This method is the most used for volume measurement, as the instruments used are commonly used in laboratories, due to its ease and wide knowledge of who performs it and because it covers a wide measurement range, from microvolumes to large capacities. When a technician is going to measure microvolume he needs to know 3 variables:

- air density, measuring environmental conditions using thermohygrometer and barometer.
- liquid density, using a pycnometer or digital densimeter.
- volume mass, using a precision balance and, if necessary, a system to prevent liquid evaporation.

The methodology for measuring the volume by the gravimetric method is described in ISO 8655-6, 2022 and consists of measuring the mass of an empty container (where the liquid will be dispensed). After setting this value, the volume previously selected on the micropipette must be dispensed into this container. Then measure the mass of this container again (now with the liquid inside). The difference between the mass value of the full container and the mass value of the empty container will be converted into volume by correcting for buoyancy and the volumetric coefficient of thermal expansion.

4. Measurements and Results

The measurement results presented in this work were obtained using a MettlerToledo balance, model XP26 PC with a resolution of 0.001 mg and a Sartorius balance, model MSA225S with a resolution of

0.01 mg. These scales have an air saturation system, which minimizes the evaporation of the volume dispensed by the micropipette and, consequently, better stability of the results.

Measurements were made using the gravimetric method at the Inmetro Fluids Laboratory using a scale with a resolution of 0.01 mg, and at the company Mettler Toledo using a scale with a resolution of 0.001 mg. In both laboratories, the environmental conditions for carrying out the calibrations were in accordance with the specifications described in ISO 8655-6.

Figure 3: **Laboratory of Volume Calibration**

It is worth mentioning the difficulty in measuring by the gravimetric method in values below 1 µL using the micropipette, which can be justified due to its construction and use. They are very small volumes and the formation of the drop that is dispensed for measurement does not happen entirely, what is formed is a fraction of the drop, which is an aggravating factor for its detachment, and which causes one of the main factors related to the non-repetition of the results. For example, when measuring with a micropipette, sometimes the liquid cannot be aspirated so easily and when dispensing, the fraction of the drop does not come off the tip. In some measurements, the non-detachment of this fraction of the drop was a reason to interrupt the calibration and start a new cycle.

Improving measurements is a key factor for results with less uncertainty. Even though these are small volumes, knowledge and metrological traceability in this range are important due to their use in different segments and the importance of having valid results.

4.1 Scale with 0.01 mg resolution - Measuring range: 2 µL

Table 1 presents the results of the calibration of the 2 µL micropipette. It is possible to observe that the uncertainty values are significantly high in the values below 1 µL. This is justified by the influence of the scale used, with the source of uncertainty regarding the calibration of the scale and its resolution. That is, having a scale with a resolution of 0.001 mg, the uncertainty of its certificate would be better, when compared to the uncertainty of the calibration of the scale with a resolution of 0.01 mg and, consequently, would not impact so much the result in the microvolume calibration in the range presented in the Table 1.

Another influence that is significant for this measurement range is related to the handling of the micropipette by the operator and is closely linked to the construction system of the equipment and its use.

Table 1: **2 µL micropipette results**

Nominal Value (µL)	Average Value	U (µL)	U (%)	*k*	ν*eff*
0.1	0.13	0.07	50.3 %	2.00	7364
0.2	0.21	0.07	31.6 %	2.00	10567
0.5	0.53	0.06	11.3 %	2.00	11860
1	0.99	0.07	7.4 %	2.00	2242
2	2.02	0.08	3.8 %	2.00	2242

Therefore, the relative influence of the uncertainty provided in the balance calibration certificate and the operator's uncertainty are very significant. As the uncertainty value of the scale calibration certificate is predefined, its influence in percentage values on the combined uncertainty will vary depending on the best measurements of the technician who performs the measurements. If they are more homogeneous, the source of uncertainty related to the operator decreases, which leads to a greater contribution of the uncertainty of the balance.

4.2 Scale with 0.01 mg resolution - Measuring range: 10 µL

Table 2 presents the results of the calibration of the 10 µL micropipette. In this measurement range, the handling of the micropipette by the operator is the source of uncertainty that has the greatest influence. The mechanical micropipette has a system for aspirating and dispensing the liquid using a piston and spring, which provides greater variability of results even with the necessary care for its use.

Table 2: **10 µL micropipette results**

Nominal Value (µL)	Average Value	U (µL)	U (%)	*k*	ν*eff*
1	1.16	0.12	10.4 %	2.00	2541
2	2.20	0.12	5.3 %	2.00	1516
5	5.11	0.20	3.8 %	2.00	1365
10	10.24	0.24	2.3 %	2.00	1422

In these measurements, the influence of the scale decreases in relation to the measurements of the 2 µL micropipette, but it is still a little significant and subject to improvement if the user wants better uncertainties.

4.3 Scale with 0.01 mg resolution - Measuring range: 100 µL

Table 3 presents the results of the calibration of the 100 µL micropipette. In this calibration, the scale used is also not the main source of uncertainty. The handling of the micropipette by the operator remains the source of uncertainty that has the greatest influence.

Table 3: **100 µL micropipette results**

Nominal Value (µL)	Average Value	U (µL)	U (%)	*k*	ν*eff*
10	10.00	0.19	1.9 %	2.00	611
20	20.16	0.32	1.6 %	2.00	577
50	49.64	0.35	0.7 %	2.00	1507
100	100.37	0.36	0.4 %	2.00	866

4.4 Scale with 0,001 mg resolution - Measuring range: 2µL

Table 4 presents the results of the calibration of the 2 µL micropipette. It is possible to verify a significant drop in the expanded uncertainty when compared with the results of the same micropipette, however measuring the scale with a resolution of 0.01 mg. Even so, the uncertainty values are still slightly high at values below 1 µL, this is justified by the influence of the handling of the micropipette by the operator and is closely linked to the constructive system of the equipment and its use, as previously explained.

Table 4: **2 µL micropipette results**

Nominal Value (µL)	Average Value	U (µL)	U (%)	*k*	ν*eff*
0.1	0.11	0.021	19.5 %	2.00	573
0.2	0.21	0.019	9.2 %	2.00	1238
0.5	0.44	0.023	5.3 %	2.00	869
1	0.95	0.023	2.4 %	2.00	1592
2	1.95	0.032	1.6 %	2.00	1542

4.5 Scale with 0,001 mg resolution - Measuring range: 10 µL

Table 5: **10 µL micropipette results**

Nominal Value (µL)	Average Value	U (µL)	U (%)	*k*	ν*eff*
5	5.18	0.065	1.25 %	2.00	979
10	10.03	0.032	0.32 %	2.00	1078

Table 5 presents the results of the calibration of the 10 µL micropipette. In these measurements, the handling of the micropipette by the operator was what most influenced the expanded uncertainty. It also had a slight influence of the repeatability of measurements in a discrete way on the source of uncertainty, as it is related to the standard deviation of measurements.

4.6 Scale with 0,001 mg resolution - Measuring range: 100 µL

Table 6 presents the results of the calibration of the 100 µL micropipette. In this calibration it was again observed the predominance of the influence referring to the handling of the micropipette by the operator and a slight influence referring to the repeatability of the measurements in a discrete way.

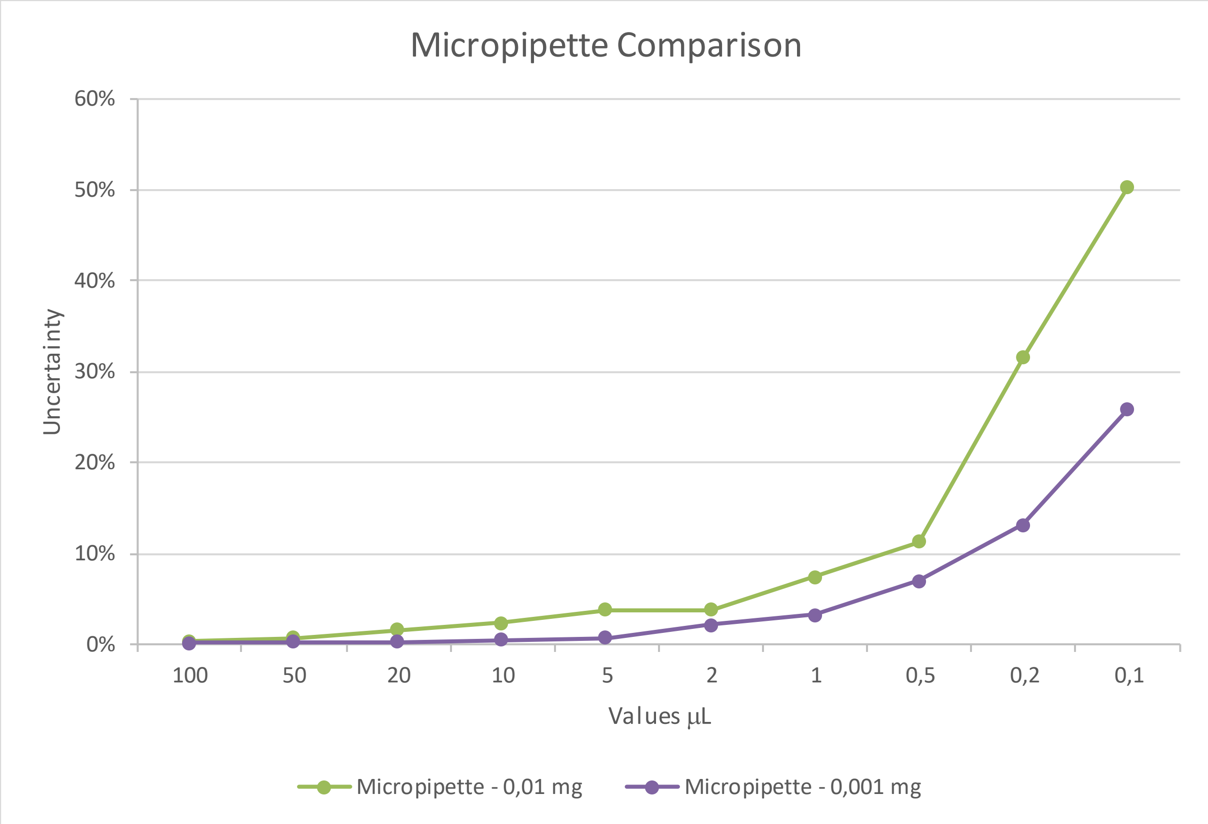
After several measurements and analysis of the results, it is possible to conclude that for calibration of the micropipette, the 100 µL point is where the scale used does not influence the expanded uncertainty of the measurement, when compared to the results using the scale with a resolution of 0.01 mg and with a resolution of 0.001 mg.

Table 6: **100 µL micropipette results**

Nominal Value (µL)	Average Value	U (µL)	U (%)	*k*	ν*eff*
20	20.90	0.065	0.31 %	2.00	1341
50	50.78	0,107	0.21 %	2.00	1094
100	101.31	0.207	0.20 %	2.00	2287

Figure 1 shows how, for values closer to 0.1 µL in micropipette calibration, the influence of the balance resolution is important and how this influence decreases as the nominal value increases. As discussed earlier, the 100 µL value is the point where the balance resolution, when compared to balances with 0.01 mg and 0.001 mg resolution, does not impact the expanded uncertainty of the measurement. From this point on, other sources of uncertainty assume greater influence.

Figure 1: **Micropipette results comparison – 0,1 µL à 100 µL**



5. Conclusion

Comparing the results shown in Tables 1, 2 and 3 with those in Tables 4, 5 and 6, it is possible to verify that for the calibration of micropipettes up to 100 µL, the use of a scale with a resolution of 0.001 mg is a fundamental factor to obtain lower uncertainty values. It is noteworthy that other factors that influence Calibration, such as operator experience, should not be neglected.

In Figure 1, this comparison is easier to observe, with all the results arranged and represented in lines. With this, it is possible to observe that from the point of 100 µL, the resolution of the balance is no longer decisive in the calculation of the expanded uncertainty, and the Calibration Laboratory must be more concerned with the other factors that become paramount.

The microvolume measurement using a micropipette makes it possible to optimize the measurements that the user makes in their daily lives, due to the simplicity of use. If more accurate results are needed, the use of a microsyringe is recommended, even knowing that its use requires greater care, takes more time and greater user skill.

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