Checking and calibrating fixed-volume and adjustable-volume piston pipettes using a gravimetric method

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Analysis and review of design solutions in terms of precision and operation ergonomics

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1. Introduction

Liquids have always been a subject of scientific and laboratory researches. It is a common knowledge that the amount of liquids during a study determines accuracy of the specific analysis. Initially pipettes were used to transfer volumes by sucking a certain amount of substance with mouth. Now it seems to be a practice of the past and this method was found dangerous since materials sucked into the pipette could reach the operator's mouth. This way researchers were exposed to absorption of hazardous chemicals, infectious organisms or radioactive materials. The first infection of this sort dates back to 1893 (Dark Daily's report) 'when a physician accidentally sucked typhoid bacilli culture'. In 1915, 47 similar cases in 57 reliable laboratories were recorded. It seems obvious that pipetting safety was crucial.

Photo 1. Dr. Adah Elizabeth Verder mouth pipetting.
Source: https://www.flickr.com/photos/nihgov/38455898272,

The testing security was and still is one of the most important aspects. For this reason the mouth pipetting is forbidden, for example in the United States of America, and rarely used in other parts of the world. What proves popular is mechanical or electronic instruments, such pipettes, burettes, diluters that have eliminated the risk of absorbing harmful substances by the user. Now volume measurements and volume transfers still play a key role in the laboratory. On the one hand you expect precision, which influences further processes, and you need to understand metrological aspects and good pipetting practice.

On the other hand precise pipetting is also an economic aspect as some substances are costly, and safety, as you know a drug is different than poison only in terms of a dose volume. The laboratory expectations and requirements related to ergonomics and analysis accuracy are constantly rising, and the response to these needs are ergonomic pipettes of a fixed and adjustable volume, offered

by Radwag. If used, they can allow quick and efficient work combined with top safety, accuracy and precision.

Photo 2. Adjustable-volume piston pipettes by Radwag

These pipettes represent a new line of 'liquid handling' products intended for quick measurement and transfer of small liquid volumes. They guarantee highly precise dosing as well as come in an ergonomic and solid design. The pipette mechanism assures unique precision and repeatability with reduced force of pressure. Their major features:

- a large high-resolution volume indicator that is fully visible during pipetting,
- an innovative soft grip that secures against heat transfer into the pipette interior,
- simple 'by click' volume change mechanism,
- possible to autoclave the entire pipette,
- resistance to UV radiation,
- compatible with most tips available on the market.

2. Types of piston pipette

The mode of operation of the piston pipette never changes, regardless of its design – the manual or automatic pressure on the piston pushes liquid out of the pipette for dosing purposes. A fixed-volume pipettes allow dosing a specific amount of liquid and it is not possible to adjust its amount. In case of excessive systematic error, it is possible to slightly adjust the amount of liquid in order to compensate for pipette errors found during calibration.

On the other hand the aforesaid minor adjustment allows adapting the pipette to precise dosage of liquid whose properties are different than water. The nominal volume of adjustable-volume pipettes shows a top limit for the volume that can be transferred. In case this range is lower, the adjustment of the volume is allowed to the extent specified by the pipette manufacturer.

Another division of pipettes is concerned with the way of collecting liquid and transferring it into the interior of the pipette. It is possible to suck the liquid in the way that it gets separated from the pipette piston (this is an A-type pipette) through the so-called 'airbag' whose volume can be referred to as pipette air dead zone. In this solution, the risk of contaminating the volume is reduced but dosing small volumes is associated with lower accuracy in view of compressibility of the air dead zone. The purpose of limiting unfavorable effects generated by air dead zone is a two-step movement of the pipette piston. In the first cycle of the piston, liquid accumulated in the pipette tip is removed while the second movement entails blow-out of air together with liquid remains that may still reside in the tip.

Photo 3. Piston pipette inspection – measuring position for XA balances and MYA microbalances

In a D-type pipette, sucked liquid has a direct contact with a pipette piston. This way the imprecision issue arising from existence of the air 'bag' is limited. This solution is dedicated to applications in which volume of transferred liquid is relatively low, e.g V < 10 µl.

Single-channel pipettes have been developed to give rise to multi-channel variants that allow simultaneous dosing of equal volumes by numerous tips. Such a working mode is a must for many biochemical and pathological laboratories, and the use of multi-channel pipettes increases researching capabilities and substantially boosts sample analysis. The growth of pipettes is so dynamic that now it is possible to gain access to a wide range of ergonomic models. Their main quality is easy use with very low operator's burden.

Aside from manually operated pipettes, there are also semi-automatic electronically controlled equivalents. Their major advantage is elimination of the so-called human factor while collecting and releasing liquids, which reduces mistakes arising from uneven liquid collection and supply. Irrespective of the pipette design, its mode of operation remains unchanged and therefore the inspection procedure is identical. It requires measuring the mass of the liquid dosed through the pipette, which allows determining the liquid volume that is equal to the pipette volume, provided the liquid density is known.

$$V = \frac{m}{\rho} \tag{1}$$

where: V volume of liquid expelled from pipette (cm^3)
m liquid mass (g)
ρ liquid density (g/cm^3)

Photo 4. Piston pipettes manufactured by Radwag

3. Normative requirements

Normative requirements for piston pipettes are specified in ISO 8655 standards – Tłokowe przyrządy do pomiaru objętości. Terminology and definitions have been showed in the part 1 of the standard, while the design and metrological properties requirements are given in the second part, i.e. ISO 8655-2 'Pipety'. The part 6 of the ISO 8655 standard gives a description of a reference procedure of gravimetric measurement adopted to determine and verify volumes of piston pipettes.

This method can be used to supervise the measuring equipment. At this point it is necessary to note that measuring equipment supervision obligation (applicable to a piston pipette) results from requirements of ISO 17025:2018-02 'Ogólne wymagania dotyczące kompetencji laboratoriów badawczych i wzorcujących – pkt 6.4 Wyposażenie' standard and requirements of PN-EN ISO 9001:2015-10 'Sytemy zarządzania jakością – pkt 7.1.5.2 Spójność pomiarowa' standard.

Regardless of normative requirements under the so-called measuring instrument life cycle, it must be periodically checked and its precision tested on the basis of traceability. Evaluation of operation of the pipette mainly applies to its metrological properties, such as correct and precise measurements, but may also extend to other fields, such as ergonomics, economical use, etc. The example of the measuring instrument life cycle has been presented in the photo 5.

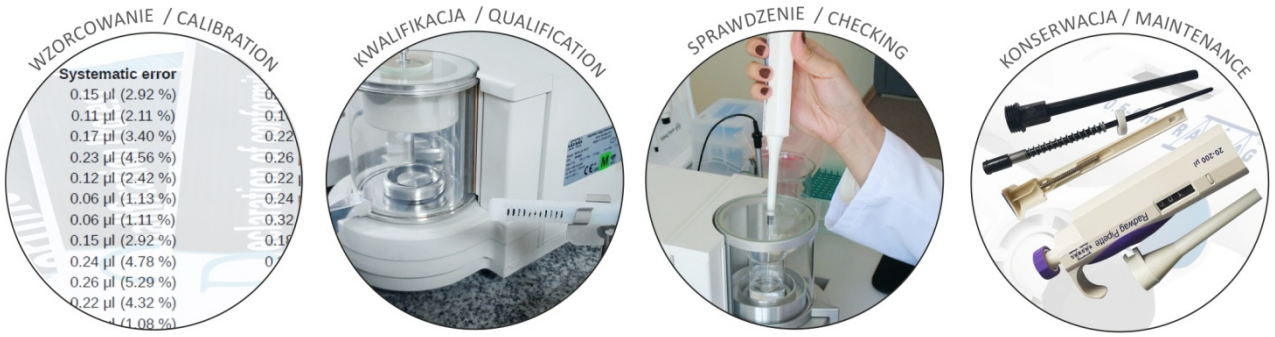


Photo 5. Piston pipette life cycle

Apart from design requirements, ISO 8655 standard specifies values for permissible limit errors that may apply to the piston pipette. These norms serve as guidelines mainly for piston pipette producers but are also used as approval criteria for piston pipette users. Similar to other measuring instruments, two error types have been defined for piston pipettes:

- es systematic error and
- CV random error.

SYSTEMATIC ERROR is a difference between an average value of the pipette volume determined from a series of at least 10 measurements and nominal value of the volume that was subject to testing. From the metrological point of view, this parameter is called correctness. The calculation method is showed in the equation 2, and 2-1.

$$e_s = \acute{V} - V_s \tag{2}$$

$$\eta_s = 100\,\% \cdot \frac{\acute{V} - V_s}{V_s} \tag{2-1}$$

where: e_s *systematic error of measurement expressed in volume units*
V_s *specific test volume of piston pipette*
η_s *relative systematic error of measurement expressed in percent*

The average volume of liquid dose that is expelled from the pipette in subsequent research cycles is defined on the basis of dependency 3.

$$\acute{V} = \frac{\sum_{i=1}^{n} V_{i,ref}}{n} \tag{3}$$

where: $\acute{V}$ *average supply volume*
$V_{i,ref}$ *every rated supply volume*
n *number of repetitions (trials)*

Photo 6. Inspecting piston pipette (200 µl of volume)

The measure of RANDOM ERROR that occurs in a series of measurements is standard deviation s_r, when this error is expressed in volume units or variability factor C, when the value of this error is given in percent.

$$s_r = \sqrt{\frac{\sum_{i=1}^{n} \left(V_i - \acute{V}\right)^2}{n-1}} \tag{4}$$

$$C_v = 100\,\% \cdot \left(\frac{s_r}{\acute{V}}\right) \tag{4-1}$$

where:s_r *standard deviation expressed in volume units*

In testing piston pipettes using the gravimetric method, the mass of expelled liquid is always recorded. For this reason it is necessary to convert weighing results into volume. It can be done in two ways. As part of the first method, you need to use a general equation (5) that allows calculating the liquid volume with special regard to the following factors:

- liquid evaporation in the cycle,
- atmospheric air density,
- standard weight density,
- water density,
- pipette thermal expansion coefficient,
- testing temperature,

$$V_{i,ref} = \left(m\middle|L - m_E + m_{evap}\right) \times \frac{1}{\rho_w - \rho_a} \times \left(1 - \frac{\rho_a}{\rho_b}\right) \times \left[1 - \gamma\left(t_w - t_{ref}\right)\right] \tag{5}$$

where:$V_{i,\mathrm{ref}}$ liquid volume at rated temperature in ml,

m_{L} weight for a weighing bottle after supplying liquid in g,

m_{E} weight for a weighing bottle before supplying liquid in g (m_{mi} = 0 for taring scale via weighing bottle)

m_{evap} estimated evaporation mass in test cycle in g,

ρ_{A} air density in g/ml during testing,

ρ_{B} standard weight density (8 g/ml),

ρ_{W} water density at test temperature (in °C) in g/ml,

γ combined cubical thermal expansion coefficient (°C−1),

t_{W} pipette temperature – assumed to be equal to test liquid temperature in °C;

t_{ref} pipette rated temperature (20°C or 27°C).

The second method is simpler because all aforesaid factors have been included in the so-called Z correcting indicator (equation 6). The liquid-to-volume mass conversion is concerned with using a suitable indicator whose value already provides for water density, atmospheric air and

temperature that the test is conducted at. The Z indicator values are given in the attachment A of the 8655-6 standard and attachment 1 to this document.

$$V_i = m_i \times Z \tag{6}$$

$$V_{i,ref} = m_i \times Z \times \left[1 - \gamma\left(t_w - t_{ref}\right)\right] \tag{6-1}$$

where: γ *combined cubical thermal expansion coefficient*
t_w *pipette temperature (usually equal to liquid temperature)*
t_{ref} *pipette rated temperature (20°C or 27₀C)*

Dependency 6-1 can be used when you know the pipette thermal expansion factor value.

A detailed description of physical dependencies that emerge while pipetting has been presented in the attachment 1 to this publication. These are scientific considerations partially used in ISO 8655 standard. Understanding of these phenomena can be helpful while evaluating the quality of piston pipettes in question and specifying areas of potential higher risk.

3.1. Design requirements for a weighing system

Balances used to check and calibrate pipettes come in a slightly different design when compared to traditional balances as the structure must provide for distinctive features of the process. The essence of measuring liquid volume expelled from the pipette is concerned with quick measurement of liquid mass at a stable temperature and higher relative humidity. As we all know, the mass measurement precision depends on numerous environmental factors and measuring cycle duration that must be as short as possible. For this reason optimization of balance structure applies to the size of the weighing pan, size of the vessel which water is released to as well as assurance of the environment in which liquid evaporation effect is minimized. The example of such a structure is showed in the photo 7.

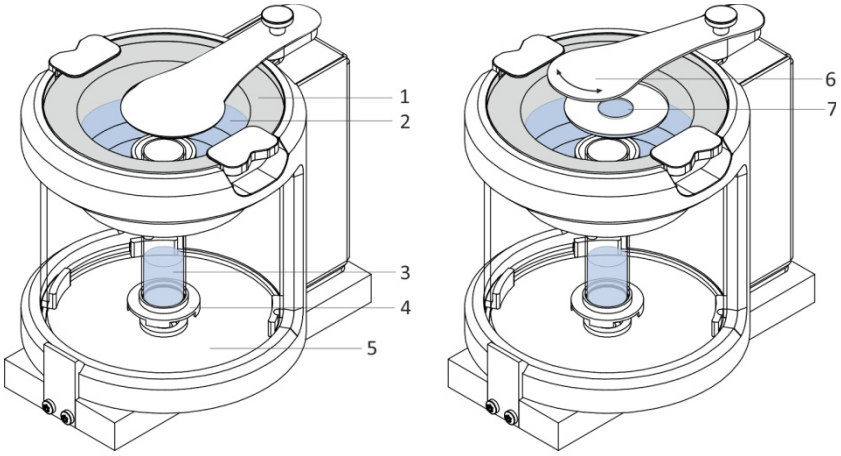


Photo 7. Automatic chamber for pipetting with the use of XA balances

In place of a typical weighing pan that the balance is supplied with, here comes a special attachment (5) with a weighing pan (4) adapted in terms of size to the vessel (3) to which the pipette liquid is expelled. The upper part of the attachment is supplied with the so-called steam curtain (1) filled with water (2). At the top of the steam curtain is a hermetic glass cover thanks to which a humidity of ca. 90% is maintained in the interior of the steam curtain. Such a solution substantially eliminates evaporation of the liquid in the vessel (3).

While liquid is dosed, the cover (6) automatically moves to uncover an injection hole – liquid from the pipette can be supplied into the weighing vessel (3). After transferring the pipette liquid into the vessel, the injection hole closes automatically and it is possible to measure the mass carefully. The installed attachment inside the XA 5Y balance has been showed in the photo 8.

Photo 8. XA balance – manual system for piston pipette volume check

The use of the steam curtain in the pipetting weighing unit complies with recommendations stipulated in the ISO 8655-6 standard in the context of liquid evaporation effect – see point 9.1 ISO 8655-6. The balance weighing chamber windows can be easily disassembled without any tools, thanks to which you can gain a direct access to the weighing vessel from all sides.

With regard to other balances, a simpler solution has been adopted. In this solution, a glass cover is attached onto the steam curtain cover. During the measurement, it must be manually moved, which is not however problematic for the personnel. Such a solution is commonly used when single-channel piston pipettes are inspected with the use of MYA microbalances – the example of such a solution is showed in the photo 9 and 10.

Photo 9. Inspecting piston pipette volume with the use of MYA microbalances

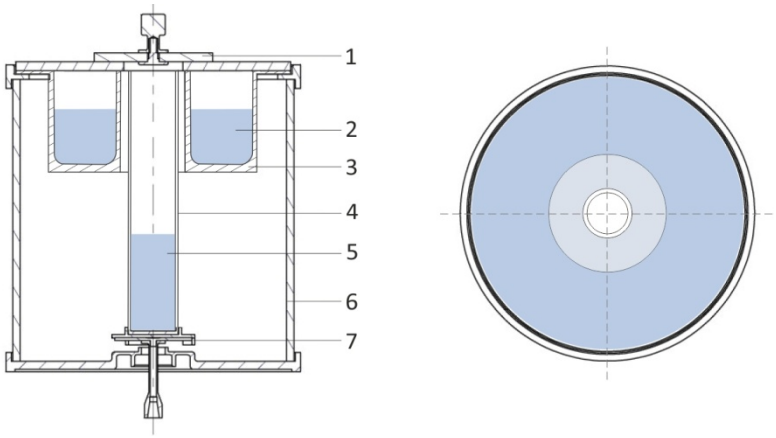


Photo 10. Steam curtain sketch for MYA microbalances and XA analytical balances

Key
1 – injection hole cover;
2 – steam curtain water
3 – steam curtain
4 – weighing vessel for releasing water from pipette
5 – water dose
6 – pipette calibration attachment
7 – weighing pan

An essential factor in the pipetting process is work ergonomics that must provide the operator with a set of measuring instruments and a measuring system design that assure quick, precise and cyclical test cycles. It is also necessary to mention a laboratory technician who should work in comfortable conditions and should not be physically overburdened. It is one of the essential factors that has a real impact on gravity of the random error that occurs in all pipetting cycles.

A high work ergonomics level is guaranteed in case of using the so-called pipette calibration stand (photo 11). This is an integrated work stand equipped with temperature sensors, humidity sensors, pressure detectors, PC software and balance (balances), which allows quick and precise evaluation of each pipette, irrespective of the volume in question.

Photo 11. Piston pipette check and calibration stand.

The above-stated design solutions are intended for single-channel pipettes, regardless of their type and structure. Checking multi-channel pipettes is possible even in case of these structures, which is confirmed in the point 8.4 b of the ISO 86655 standard. This process is however ineffective and time-consuming, and proves uneconomical.

While multi-channel pipettes are checked, automatic systems are used to simultaneously supply liquid from each channel to dedicated containers.
In the measuring cycle, automatic taring occurs, followed by weighing of each empty container or container with liquid. A net weight of the liquid for each channel is calculated as a difference of the container mass before liquid dosage and after releasing the liquid from each channel of the pipette into the container. Such a solution shortens the multi-channel pipette testing time at least a few times. The measuring system intended to inspect multi-channel pipettes is showed in the photo 12 and 13.

Photo 12. AP 12.5Y automatic system for testing multi-channel pipettes

Automation of the piston pipette check and calibration provides new benefits, particularly while assessing precision of the device operation (operating qualification). In the manual mode, every test requires intervention of the operator who must use standard weights to establish relations between the balance readout and weight value – this is the balance systematic error. By monitoring dispersion of measuring results, it is possible to estimate the random error value for the balance. It must be noted that both tests are conducted with the use of standard weights while the pipette volume check applies to the water mass measurement. It is assumed that there are no substantial metrological differences between these measurements, mass standard – water.

In the automatic system, determination of the systematic error value for the balance also requires the use of standard weights, but evaluation of the random error can be performed in the automatic cycle.

Precision of measurements can be determined with the use of an Autotest function that is available to the operator. This function is concerned with cyclical movement of each container (water reservoirs) from the base spot into the mass measurement stand. The number of measuring cycles can be specified, and therefore in the aftermath of the procedure, AP 12.5Y exposes values of the standard deviation for each container. This is a measure of precision used to weigh water released from the multi-channel pipette during real measurements – verification, calibration. Such a test must be carried out under machine operating qualification (i.e. AP 12.5Y) without water. Supplying a small amount of water into the container interior leads to liquid evaporation which distorts the result of the test whose goal is to check precision of the automatic weighing. This is a more realistic approach to evaluation of precision that is one of the most important metrological parameters of each device. It is certainly possible to modify this research method in terms of assessing the steam curtain efficiency. The main components of the AP 12.5Y automatic unit have been showed in the photo 13.

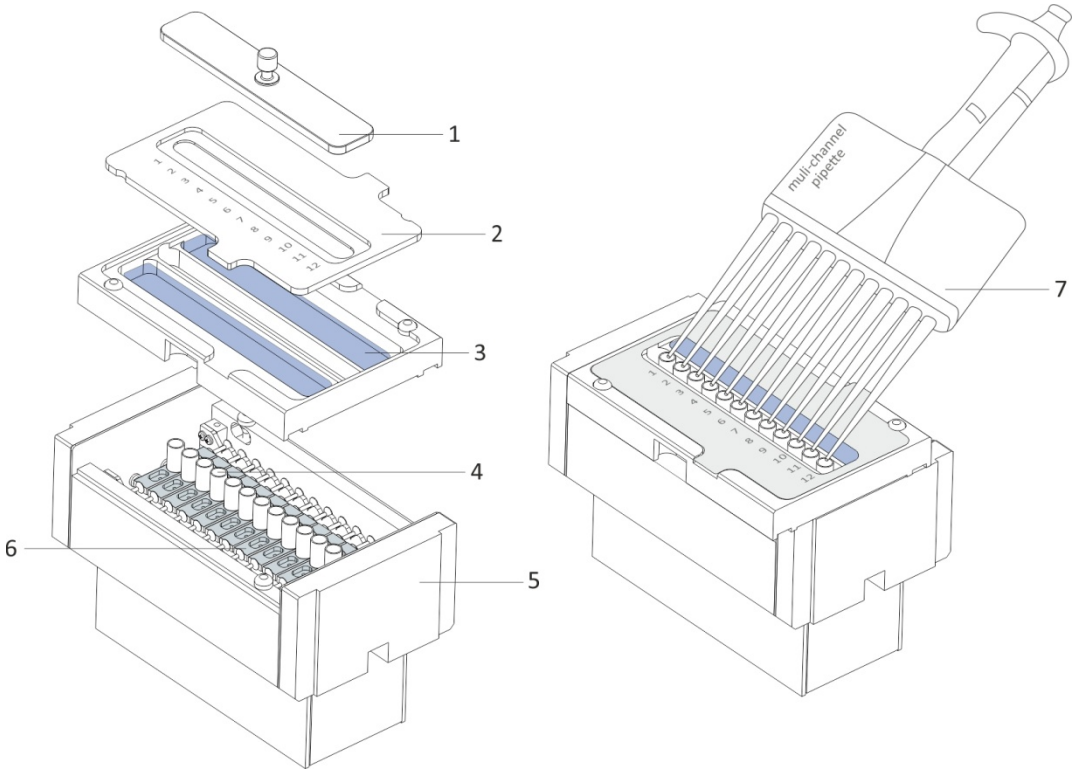


Photo 13. Components and design of the AP 12.5Y automatic unit

Key:
1 – measuring line cover
2 – steam curtain cover
3 – steam curtain
4 – container injection hole
5 – container conveyor
6 – expelled liquid container
7 – multi-channel pipette

A key element that influences the testing precision is stability of the balance installed in the AP 12.5Y system. This is a microbalance unit whose maximum load is 52 g with an elementary reading unit of d = 0.01 mg. In the second variant, the maximum load of the measuring unit is 21 g and elementary reading unit of d = 0.01 mg. This solution has been additionally supplied with an outer measuring line cover for prevention of the weighing procedure against negative external factors, mainly air movement.

The operation of running gear of the container conveyor in each model has been optimized in terms of speed and high precision of testing. All measurements are stored in the database of the AP 12.5Y automatic unit. Once the testing is over, a pipette testing report is generated. Another solution is a direct transfer of liquid dose mass measurement into the client's external application. This information is verified there. The weighing result can be sent through the cable (RS 232, Ethernet) or remotely via WiFi, FreeLink.

3.2. Mass measurement and weighing unit volume precision

When it comes to measuring low weights* (liquid, solid), the only essential parameter that influences quality/precision of the analysis is measurement precision. Precision is usually expressed through measures of imprecision showing the gravity of measurement dispersion in the series. As a rule this is a standard deviation that serves as the most significant metrological parameter during piston pipette check and calibration. It must be noted that the average value of the pipette volume used to evaluate the systematic error (equation 2) can be of the same value for the series of measurements of a low dispersion and series whose results are extremely dispersed. For this reason balances designed to check piston pipettes have been assigned limit values of the standard deviation. Other metrological factors, such as linearity error, centricity error are insignificant, yet may be considered in the uncertainty budget. Depending on rated volume of the pipette, the measurement precision requirements are showed in the table 1.

*) – low weight is a weight value lower than the design limit which for electronic balances is defined as 5% of the maximum load of the balance (m < 5 % Max).

Table 1. Minimum requirements for balances

Instrument rated volume (V)	**Elementary reading unit** (d)	**Repeatability** (s)[a]	**Expanded uncertainty of measurement** U (k = 2)[a, b]
	mg	mg	mg
0,5 µl ≤ V < 20 µl	0.001[c] 0.1[d]	0.006[c,e] 0.03[d]	0.012[c e] 0.06[d]
20 µl ≤ V < 200 µl	0.01	0.025	0.05
200 µl ≤ V ≤ 10 ml	0.1	0.2	0.4
10 ml < V ≤ 1 000 ml	1	2	4
1 000 ml < V ≤ 2 000 ml	10	10	40

a – repeatability and measurement expanded uncertainty values given in this table apply to determination of the single-channel pipette volume. When the single-channel balance is used only to determine volume in multi-channel pipettes, repeatability and measurement expanded uncertainty values are twice as high as values entered in this table.

b – the measurement expanded uncertainty can be estimated on the basis of EURAMET cg-18, ver. 4.0 or ASTM E898 conductors for the rated volume. The measurement expanded uncertainty covers non-corrected errors, as well as potential drift and environmental impact on the balance sensitivity. The measurement expanded uncertainty can be derived from the balance calibration certificate or calculated separately.

c – single-channel balance.

d – a multi-channel balance, e.g. AP 12.5Y by Radwag, is suitable for testing multi-channel pipettes. Multi-channel pipettes with an elementary reading unit of 0.01 mg can be used for multi-channel pipette trials if the rated volume is below 20 µl only when the measurement expanded uncertainty is lower than the one fourth of the maximum permissible systematic error for the pipette.

e – with regard to single-channel pipettes whose rated volume is below 2 µl, it is necessary to use the balance whose repeatability and expanded uncertainty is better than values entered in the table. Requirement: expanded uncertainty must be lower than one fourth of the maximum permissible systematic error of the pipette.

Importantly, precision of the measurement is a fixed feature of every balance – in ideally permanent working conditions, without essential interference, the precision is stable. Unfortunately surrounding conditions vary and measurements are disturbed through the operator's work (strokes, lack of repeatable cycles), liquid sorption and desorption, etc. You cannot forget that precision of measurements is considerably affected also by precision of releasing liquids from the pipette; after all a pipette is a measuring tool too. Potential sources of errors that may occur while pipetting are showed in the photo 14.

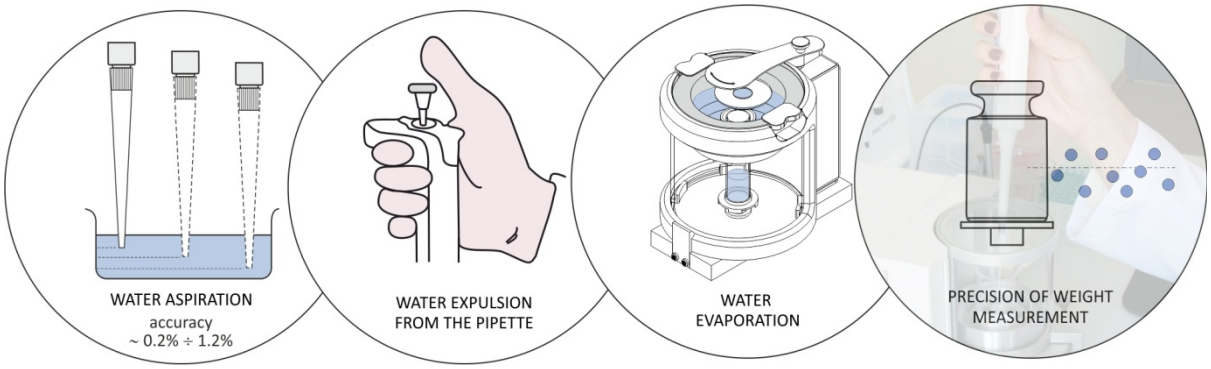


Photo 14. Sources of errors while pipetting

4. Piston pipette tips

It is advisable to use pipette tips recommended by the manufacturer. It will allow minimizing measurement error risk and making sure that they are suitable for the pipette and have a relevant internal volume. It must be noted that potential contaminants can be transferred from the sample to the pipette, from the pipette to the sample and from the sample to the sample. Considering the first scenario, the liquid or its aerosols are transferred into the pipette cone, mainly due to operator's mistake. Transferring contaminants from the pipette to the sample essentially results from absorption of aerosols, e.g. minor fractions of liquid or dust accumulated in the pipette cone. A contaminant may attach to the inner surface of the cone and can be removed only during subsequent pipetting cycles.

Another, yet more advanced, variant is filter-rich tips (fig. 15) that come in handy when radioactive materials, infectious materials are used or when cross contamination risk is possible. This solution helps in eliminating problems related to transferring contaminants into the pipette structure. In other solutions, displacement pipettes are equipped with a piston and sealing that prevent contaminants from penetrating the interior of the pipette.

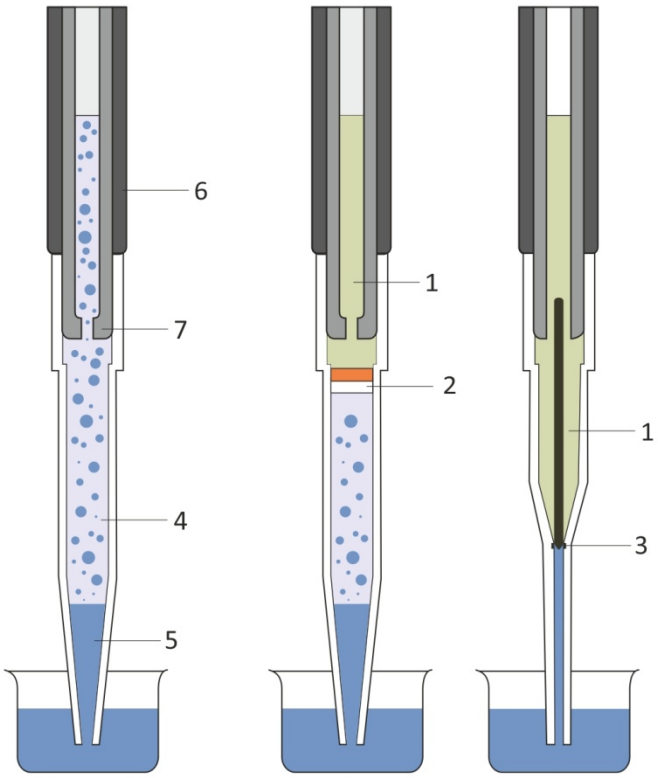


Photo 15. Liquid collection for various pipettes

With regard to displacement pipettes (A type), a tip of the pipette (usually made of plastic) is attached onto the pipette cone and, after transferring the volume, removed with no attempts to clean it. There are also other tips, made of metal, glass, Teflon-coated plastic. They can be easily cleaned using ultrasonic methods, for instance.

Key
1 – safe area
2 – filter
3 – pipette piston edge protection
4 – unsecured area (pollution transfer possible)
5 – liquid dose supplied to pipette
6 – pipette
7 – pipette cone

5. Working conditions in the Laboratory

The work ergonomics in the laboratory must be assured even when you do not intend to perform numerous repeatable tests. Ergonomics is a very general term but must be understood as a location, structure, equipment and size of the working stand that provides stable working conditions, minimizes the operator's burden and lowers the mistake risk (photo 14).

The measuring element used to check piston pipettes is a balance. Therefore what is expected is constant ambient temperature, stable humidity, lack of substantial vibrations of the floor and lack of excessive air movement. The ambient temperature stability is also crucial for the pipette and tips in view of the thermal expansion and heat transfer. It must be noted that the recommended stabilization and testing temperature of 20°C can be hard to reach in many laboratories. The use of a traditional wall-mounted air conditioner always results in excessive air movement, which substantially hinders mass measurement. In effect pipette testing may be erroneous.

Photo 16. Various-volume piston pipette checking stand

The best solution is the so-called laminar air-flow air conditioning as the air is sucked from the bottom through several air grates. Next it is transported into a dispersion unit, usually installed in the ceiling or above the ceiling of the laboratory. Inside the system, the air is cleansed, moisturized, heated or cooled.

6. Liquid collection and dosage

Although it looks relatively simple, a pipetting process is complicated as it requires the operator to take actions which eventually assure smooth and regular operation of the pipette piston. It must be remembered that usually tests entail a series of ca. 10 repetitions and the measure of accuracy and precision of the pipette is the average value or standard deviation from the series of measurements. Please remember that the pipetting methodology also involves such elements as a method of removing the pipette tip from the vessel, pipette tip immersion depth, pipette immersion in liquid time, etc.

As a rule the pipette must be positioned vertically while sucking the liquid. Deviations from this rule may lead to imprecision due to various liquid pressure in the pipette tip. Another important parameter is a tip immersion depth, as this value may vary depending on the size, type and brand of the pipette – manufacturer's recommendations must be checked. When such recommendations are unavailable, you can follow general guidelines showed in the table 2.

Table 2. Pipette tip immersion depth, depending on nominal pipette volume

Pipette volume (µl)	Pipette tip immersion depth (mm)	Waiting time (sec.)
≤ 1	1 ÷ 2	1
> 1 ÷ 100	2 ÷ 3	1
> 100 ÷ 1 000	2 ÷ 4	1
> 1 000 ÷ 5 000	3 ÷ 5	3

It was experimentally confirmed that repeated initial moisturization of the pipette tip may help you keep precision of the pipette because it limits air bubbles. Initial moisturization also stabilizes the dead zone of the pipette air volume between the liquid and pipette piston. The aforesaid moisturization is a must when the hydrophobic or high-viscosity liquids are transferred. It is very important in the case of high pressure of steam. With respect to displacement pipettes whose volume is below ca. 10 µl, the initial moisturization is not required (Blues, Bayliss & Buckley - Measurement Good Practice Guide No. 69. The Calibration and Use of Piston Pipettes).

After collecting the liquid, drops that adhere to its tip must be carefully removed. If you can still see the surplus of liquid that adheres to the outer surface of the tip, you can carefully remove it with a suitable materials, making sure you do not contaminate the liquid. When subsequent drops accumulate on the pipette tip, it may suggest a badly fitted tip or dead air volume instability, particularly if the liquid is known for a high steam pressure. Wrong collection of the liquid may generate substantial errors, as showed in the photo 17.

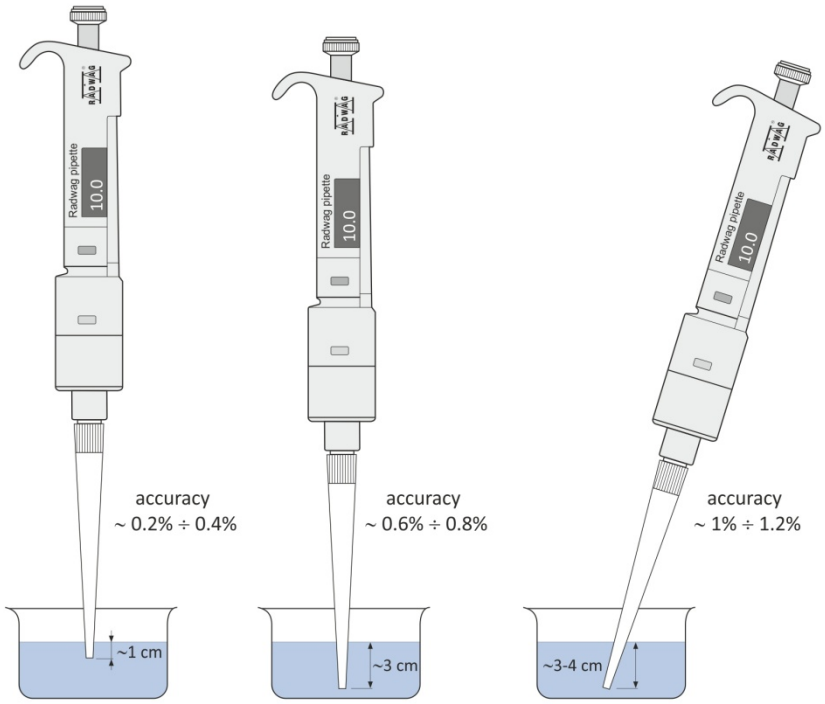


Photo 17. Potential measurement mistakes while collecting water

Liquid dosage requires the operator to gently touch the wall of the vessel with the pipette tip just above the liquid surface at the angle of about 30° to 45° and then move the tip vertically upwards along the inner wall of the vessel at the height of 8-10 mm when dosing is finished.

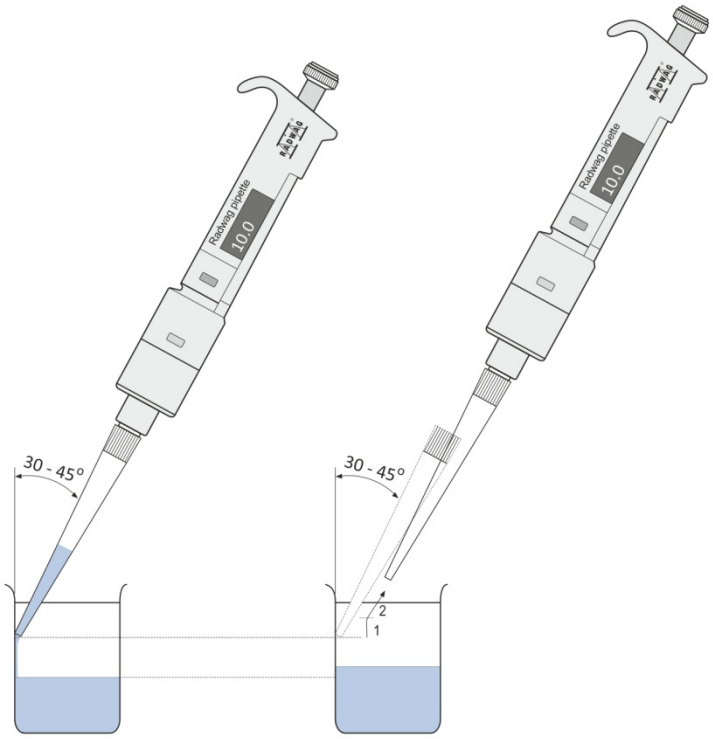


Photo 18. Method of expelling liquid from pipette

7. Research part

The most important metrological parameter of each balance used to inspect the piston pipette volume is repeatability of results whose measure is a standard deviation. With respect to balances dedicated to single-channel pipettes, evaluation of measurement precision was concerned with the process in which the weighing pan was cyclically loaded with the same standard weight. Such a method of evaluating the measurement precision does not provide for mistakes generated by the operator testing the pipette (strokes, air bubbles, pipette inclination angle, etc.). It is assumed that the impact of the so-called human error can be substantially limited through training. Importantly the balance expanded uncertainty as a measure of utility/correct adaptation of balance to volume of the pipette in question involves other factors, such as balance drift, liquid evaporation, etc. The research part of the balances dedicated to single-channel pipettes includes assessment of result repeatability (table 3) and dynamics related to liquid evaporation from the weighing vessel. These are two factors that are virtually beyond the operator's control.

As for the AP 12.5Y automatic system, the mass of the liquid in question is calculated on the basis of difference of masses of the container before and after injection. The consequence of this procedure is a double measurement of the container mass, which is not favorable from the metrological point of view. At the first stage precision was specified for measurements of point mass, that is inner adjustment mass. The purpose of this testing was to determine whether the measuring unit works properly. The second stage entailed inspecting the precision of weighing all containers. A variable factor in this test was a dynamic movement of the running gear which together with cyclical further containers led to minor strokes. The last step was to determine the impact of lack of steam curtain on the mass measurement – dynamics related to liquid evaporation from the weighing vessel.

7.1. Selection of balance for procedure

Selection of the balance for the piston pipette calibration and checking is primarily concerned with balance result repeatability. This is obligatory and specified in the ISO 8655-6 standard, see table 1. In addition to the aforementioned repeatability, another important value is expanded uncertainty that must be referred to the value of maximum systematic errors of the pipette. The table 3 shows requirements stipulated in ISO 8655-6 and adapted to particular types of the balance, which may be of support for operators who design their own measuring stand.

Table 3. Selection of balance for volume of the piston pipette.

	Max. load	Elementary reading unit	Repeatability of stand. dev.	Pipette volume
MYA 21.5Y.P[1)]	21g	d=1μg	S=1.0μg	1 μl ≤ V ≤ 10 μl 10 μl < V ≤ 100 μl 100 μl < V ≤ 1000 μl 1 ml < V ≤ 10 ml
XA 6/21.5Y.M.A.P[2)]	6/21g	d=1/2μg	S=1.3μg	
XA 21.5Y.M.A.P[2)]	21g	d=1μg	S=1.3μg	
XA 21/52.5Y.M.A.P[2)]	21/52g	d=1/5μg	S=1.5μg	
XA 53.5Y.M.A.P[2,3)]	52g	d=1μg	S=1.5μg	
XA 52.5Y.M.A.P[2,3)]	52g	d=5μg	S=2.2μg	10 μl < V ≤ 100 μl 100 μl < V ≤ 1000 μl 1 ml < V ≤ 10 ml
XA 82/220.5Y.A[4)]	82/220g	d=0.01/0.1mg	S=5μg	
XA 120/250.5Y.A[4)]	120/250g	d=0.01/0.1mg	S=5μg	

1) – cooperation with the MY11 pipette calibration attachment
2) – cooperation with the XA11 pipette calibration attachment
3) – cooperation with the XA17 pipette calibration attachment
4) – cooperation with the XA100 pipette calibration attachment

7.2. Precision of measurement of the AP 12.5Y / AP 12.1.5Y automatic unit

The precision of measurements for AP 12.5Y has been determined on the basis of the automatic method that required cyclical weighing of each containers located in the position 1-12 (Autotest function). Balance indication had been zeroed only before a series of measurements was initiated. Results of the precision of measurements are showed in the photo 19.

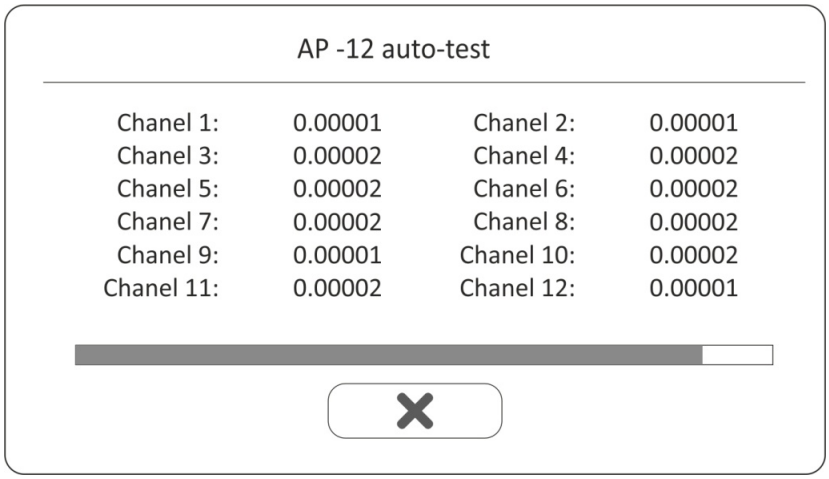


Photo 19. Results from the automatic control procedure – standard deviation from 10 measurements

Precision of measurements of the container mass measured in the automatic cycle ranges from 0.01mg to 0.02mg. It complies with requirements of the ISO 8655-6 standard.

With regard to the AP 12.1.5Y automatic unit with elementary reading unit of d = 1 μg, the precision of mass measurement was determined using the semi-automatic method – subsequent containers were moved into the weighing stand by means of the service application. This mode was used to perform 6 measuring series. The time required to obtain a stable measurement result for a single weighing procedure was ca. 10 seconds.

Table 4. Precision of mass measurement - AP 12.1.5Y, elementary reading unit - d = 1μg

Cont. no.	series 1	series 2	series 3	series 4	series 5	series 6	$\acute{x}$	S (μg)
1	9,229802	9,229802	9,229796	9,229797	9,229800	9,229797	9,229799	2,68
2	9,263251	9,263250	9,263244	9,263248	9,263246	9,263244	9,263247	2,99
3	9,257741	9,257741	9,257736	9,257736	9,257738	9,257736	9,257738	2,45
4	9,256452	9,256449	9,256444	9,256441	9,256446	9,256443	9,256446	4,07
5	9,283330	9,283327	9,283324	9,283321	9,283326	9,283326	9,283326	3,01
6	9,234612	9,234610	9,234608	9,234603	9,234609	9,234608	9,234608	3,01
7	9,230830	9,230823	9,230822	9,230818	9,230822	9,230824	9,230823	3,92
8	9,216691	9,216687	9,216688	9,216682	9,216686	9,216689	9,216687	3,06
9	9,235992	9,235988	9,235987	9,235981	9,235989	9,235987	9,235987	3,61
10	9,197869	9,197863	9,197864	9,197858	9,197865	9,197864	9,197864	3,54
11	9,300828	9,300822	9,300823	9,300821	9,300822	9,300823	9,300823	2,48
12	9,245068	9,245059	9,245062	9,245057	9,245062	9,245061	9,245062	3,73

The precision of measuring containers mass ranges from 2.45μg to 4.07μg. These values are slightly higher than the ones obtained while testing the same parameter with a point mass. The essential difference between testing methods applies to the point of suspension of the center of gravity of the mass in question.

With regard to the external weight or internal adjustment mass, the center of gravity of the item in question is relatively low. In view of the nature of measurements, the center of gravity of the container which liquid has been poured into is a way higher. This is unfavorable for forces that emerge during a weighing procedure (photo 20).

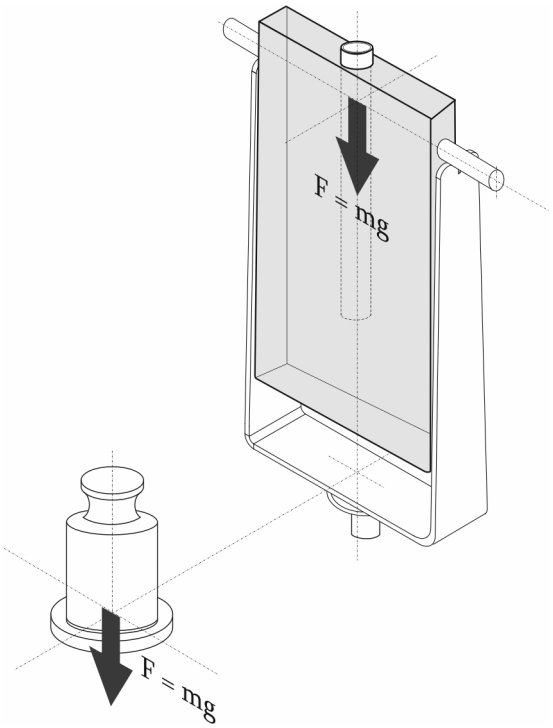


Photo 20. Container weighing procedure

7.3. Operating speed of the AP 12.5Y automatic unit

The main advantage of automation in any field is rise in speed and efficiency of work, which applies to the AP 12.5Y system. This solution assures optimization of the device in mechanical, electronic and metrological terms. Specification of the weight of water dosed for a 12-channel pipette requires precise measurement of mass of each container (each dose of liquid). In so doing, the so-called buffering mechanism has been used. Thanks to this, a suitable working speed with precise measurement have been obtained. The measuring time for a single container is ca. 5 seconds, while the total time required to specify the dose mass of the 12-channel pipette is ca. 85 seconds. While testing the 12-channel pipette as per requirements of the PN-EN ISO 8655-6:202 standard in the cycle of 10 measurements for 3 volumes, the total testing time is ca. 50-55 minutes after taking into consideration 30 doses of water and software use.

7.4. Precision of measurements in the automatic cycle

Precision of weighing in the automatic mode has been checked by specifying the difference between the container mass recorded while taring and container mass while weighing them. It was assumed that a perfectly operating system adopted a value of the container mass that was the same or negligibly different than the tare value. This test did not involve the steam curtain and containers were perfectly dry. Such an approach was necessary in order to eliminate mass drifts arising even when the small amount of water accumulated inside the container.

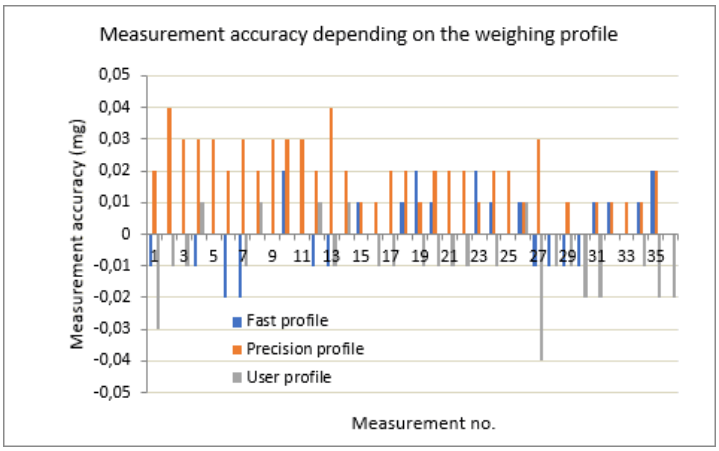


Photo 21. Precision of measuring container mass in the automatic cycle

Irrespective of the selected weighing profile, the mass of the dosed liquid can deviate by 0.02 mg at the most when Fast weighing profile is used, and by 0.02-0.02 mg in case of User profile. Slightly worse results apply to the Precision profile. A default profile for controlling pipettes is Fast.

7.5. Steam curtain of MYA microbalances and XA balances

Measuring a mass of samples that prove susceptible to sorption or desorption of moisture is always challenging because it is difficult to elaborate the research method that remains resistant to such a phenomenon. A special case is liquid mass measurement, especially when the elementary reading unit of the scale is 1 µg. Even an enclosed weighing system always aims to

achieve balance in terms of humidity and pressure. In case of lack of a steam curtain during a liquid dose weighing procedure, a dynamic change occurs, as showed in the photo 22.

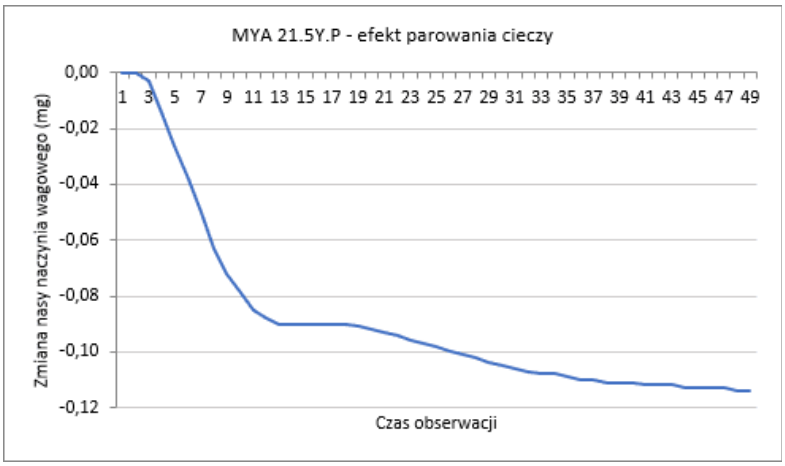


Photo 22. MYA 21.5Y.P – liquid evaporation effect

Photo 23. MYA 21.5Y.P microbalance with steam curtain.

7.6. Steam curtain of the AP 12.5Y automatic system

Limited evaporation of liquid from the weighing vessel is crucial to obtain a correct result for a specific pipette volume. With regard to single-channel pipettes, the water dose mass measurement is relatively quick, so the mass variability phenomenon does not need to be significant. When the analysis applies to a multi-channel pipette, only the first dose from the first channel is weighed immediately. The higher the number of the pipette channel, the longer the waiting time for mass measurement. For this reason the steam curtain in the automatic unit and

structure of the weighing vessel must limit evaporation process as much as possible. The photo 24 shows the difference applicable to the operation without the steam curtain and with the steam curtain.

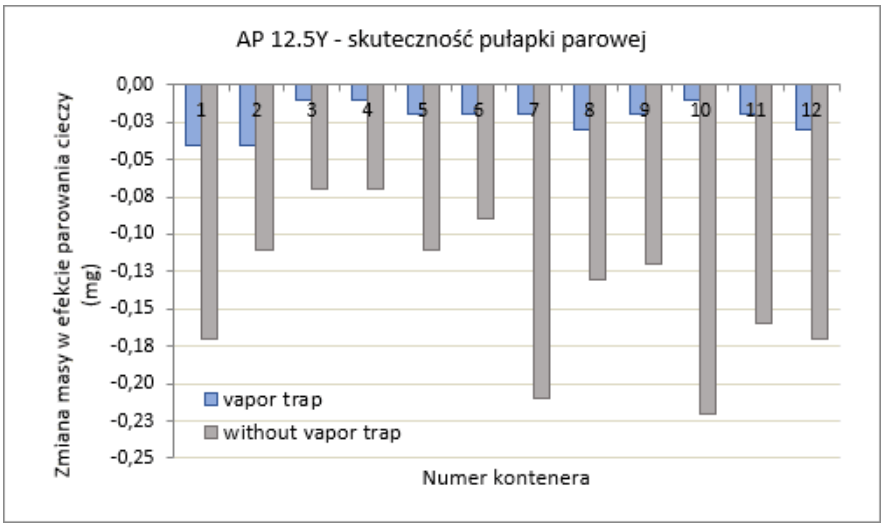
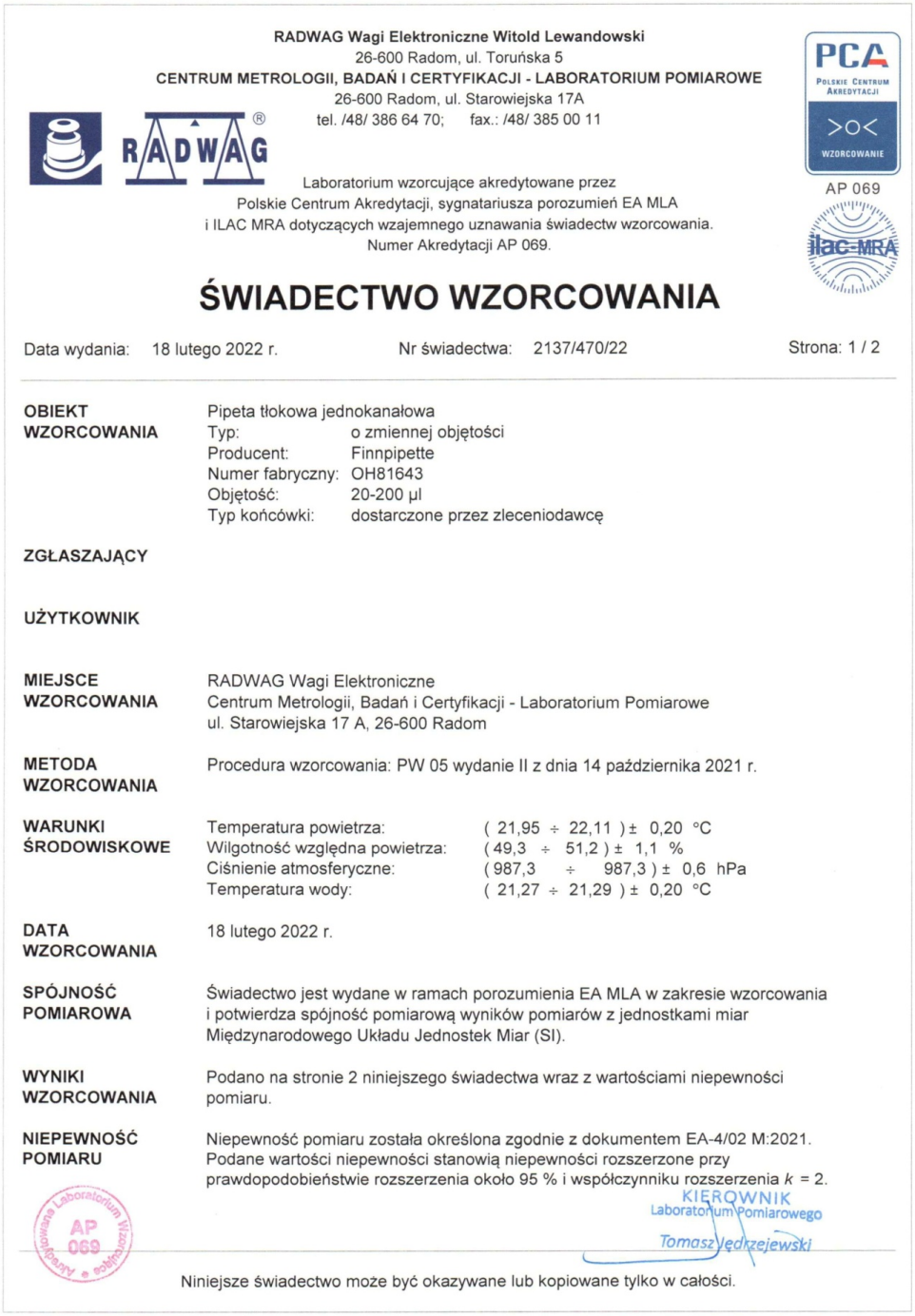


Photo 24. Liquid evaporation from the weighing vessel in the AP 12.5Y system

The change of mass of containers with liquid is only ca. 0.02mg-0.03mg but you need to remember that this value is subject to result repeatability error. For this reason you can assume that the evaporation effect is a way weaker. In case of lack of a steam trap, liquid evaporation is at least a few times higher and ranges from 0.10mg to 0.20mg. This quick testing shows how important it is to assure optimal conditions during pipetting.

7.7. Piston pipette calibration



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Laboratorium wzorcujące akredytowane przez
Polskie Centrum Akredytacji, sygnatariusza porozumień EA MLA
i ILAC MRA dotyczących wzajemnego uznawania świadectw wzorcowania.
Numer Akredytacji AP 069.

AP 069

ŚWIADECTWO WZORCOWANIA

Data wydania: 18 lutego 2022 r. Nr świadectwa: 2137/470/22 Strona: 1 / 2

OBIEKT WZORCOWANIA
Pipeta tłokowa jednokanałowa
Typ: o zmiennej objętości
Producent: Finnpipette
Numer fabryczny: OH81643
Objętość: 20-200 µl
Typ końcówki: dostarczone przez zleceniodawcę

ZGŁASZAJĄCY

UŻYTKOWNIK

MIEJSCE WZORCOWANIA
RADWAG Wagi Elektroniczne
Centrum Metrologii, Badań i Certyfikacji - Laboratorium Pomiarowe
ul. Starowiejska 17 A, 26-600 Radom

METODA WZORCOWANIA
Procedura wzorcowania: PW 05 wydanie II z dnia 14 października 2021 r.

WARUNKI ŚRODOWISKOWE

Temperatura powietrza:	(21,95 ÷ 22,11) ± 0,20 °C
Wilgotność względna powietrza:	(49,3 ÷ 51,2) ± 1,1 %
Ciśnienie atmosferyczne:	(987,3 ÷ 987,3) ± 0,6 hPa
Temperatura wody:	(21,27 ÷ 21,29) ± 0,20 °C

DATA WZORCOWANIA
18 lutego 2022 r.

SPÓJNOŚĆ POMIAROWA
Świadectwo jest wydane w ramach porozumienia EA MLA w zakresie wzorcowania i potwierdza spójność pomiarową wyników pomiarów z jednostkami miar Międzynarodowego Układu Jednostek Miar (SI).

WYNIKI WZORCOWANIA
Podano na stronie 2 niniejszego świadectwa wraz z wartościami niepewności pomiaru.

NIEPEWNOŚĆ POMIARU
Niepewność pomiaru została określona zgodnie z dokumentem EA-4/02 M:2021. Podane wartości niepewności stanowią niepewności rozszerzone przy prawdopodobieństwie rozszerzenia około 95 % i współczynniku rozszerzenia $k = 2$.

KIEROWNIK
Laboratorium Pomiarowego
Tomasz Jędrzejewski

Niniejsze świadectwo może być okazywane lub kopiowane tylko w całości.

Photo 25. Single-channel pipette calibration certificate

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METROLOGY, RESEARCH AND CERTIFICATION CENTER – MEASURING LABORATORY

26-600 Radom, ul. Starowiejska 17A

tel. 48 386 64 70; fax. 48 385 00 11

The calibration laboratory accredited by Polish Center for Accreditation, a signatory of EA MLA and ILAC MRA agreements on mutual acknowledgment of the calibration certificates.

Accreditation number AP 069.

CALIBRATION CERTIFICATE

Date of issue: 18 February 2022	Certificate no.: 2137/470/22	Page: 1/2
CALIBRATED ITEM	Single-channel piston pipette	
	Type: adjustable-volume	
	Manufacturer: Finnpipette	
	Serial number: OH81643	
	Volume: 20-200 µl	
	Tip type: supplied by the ordering party	
REPORTING PARTY		
USER		
CALIBRATION VENUE	RADWAG Wagi Elektroniczne	
	Metrology, Research and Certification Center – Measuring Laboratory	
	ul. Starowiejska 17A, 26-600 Radom	
CALIBRATION METHOD	Calibration procedure: PW 05 issue II as of 14 October 2021	
ENVIRONMENTAL		
CONDITIONS	Air temperature: (21.95 ÷ 22.11) ± 0,20 ºC	
	Relative air humidity: (49.3 ÷ 51.2) ± 1.1%	
	Atmospheric pressure: (987.3 ÷ 987.3) ± 0.6 hPa	
	Water temperature: (21.27 ÷ 21.29) ± 0.20 ºC	
CALIBRATION DATE	18 February 2022	
MEASUREMENT		
TRACEABILITY	The certificate is issued under EA MLA agreement with regard to calibration and confirms the measurement traceability of results with units of measure of the International System of Units (SI).	

CALIBRATION RESULTS	Given in the page 2 of this certificate together with measurement uncertainty values.
MEASUREMENT UNCERTAINTY	The measurement uncertainty was specified as per EA-4/02 M:2021 document. Uncertainty values serve as expanded uncertainties at expansion probability of about 95% and expansion coefficient k=2.

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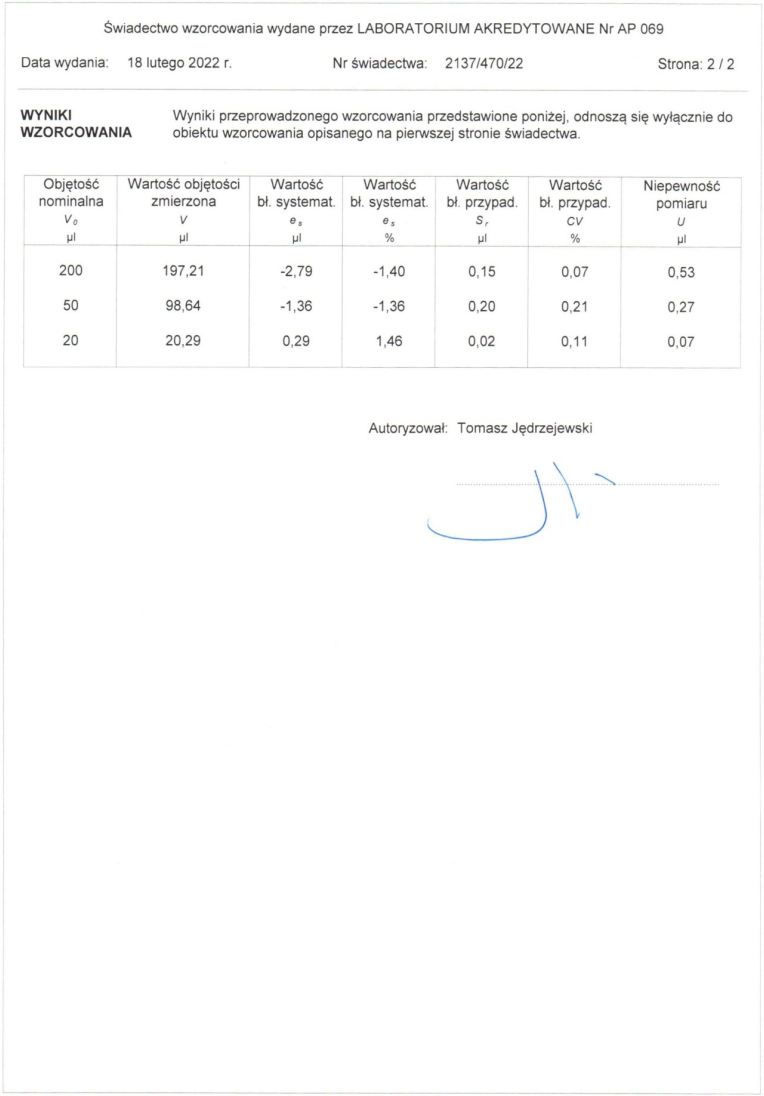
This certificate can be presented or copied in full only.



Świadectwo wzorcowania wydane przez LABORATORIUM AKREDYTOWANE Nr AP 069

Data wydania: 18 lutego 2022 r. Nr świadectwa: 2137/470/22 Strona: 2 / 2

WYNIKI WZORCOWANIA Wyniki przeprowadzonego wzorcowania przedstawione poniżej, odnoszą się wyłącznie do obiektu wzorcowania opisanego na pierwszej stronie świadectwa.

Objętość nominalna V_0 µl	Wartość objętości zmierzona V µl	Wartość bł. systemat. e_s µl	Wartość bł. systemat. e_s %	Wartość bł. przypad. S_r µl	Wartość bł. przypad. CV %	Niepewność pomiaru U µl
200	197,21	-2,79	-1,40	0,15	0,07	0,53
50	98,64	-1,36	-1,36	0,20	0,21	0,27
20	20,29	0,29	1,46	0,02	0,11	0,07

Autoryzował: Tomasz Jędrzejewski

Photo 26. Single-channel pipette calibration certificate - results

CALIBRATION RESULTS Results of the calibration, showed below, refer only to the calibrated item described in the first page of this certificate.

Nominal volume V_0 µl	Measured volume value V µl	Systematic error value e_s µl	Systematic error value e_s %	Random error value S_r µl	Random error value CV %	Measurement uncertainty U µl
200	197.21	-2.79	-1.40	0.15	0.07	0.53
50	98.64	-1.36	-1.36	0.20	0.21	0.27
20	20.29	0.29	1.46	0.02	0.11	0.07

Approved by: Tomasz Jędrzejewski

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Laboratorium wzorcujące akredytowane przez
Polskie Centrum Akredytacji, sygnatariusza porozumień EA MLA
i ILAC MRA dotyczących wzajemnego uznawania świadectw wzorcowania.
Numer Akredytacji AP 069.

AP 069

ŚWIADECTWO WZORCOWANIA

Data wydania: 18 lutego 2022 r. Nr świadectwa: 2136/470/22 Strona: 1 / 2

OBIEKT WZORCOWANIA	Pipeta tłokowa ośmiokanałowa	
	Typ:	o zmiennej objętości
	Producent:	Eppendorf
	Numer fabryczny:	J38259G
	Objętość:	10-100 µl
	Typ końcówki:	dostarczone przez zleceniodawcę
ZGŁASZAJĄCY		
UŻYTKOWNIK		
METODA WZORCOWANIA	Procedura wzorcowania: PW 05 wydanie II z dnia 14 października 2021 r.	
WARUNKI ŚRODOWISKOWE	Temperatura powietrza:	(21,73 ÷ 21,78) ± 0,20 °C
	Wilgotność względna powietrza:	(48,8 ÷ 50,2) ± 1,2 %
	Ciśnienie atmosferyczne:	(986,5 ÷ 995,1) ± 0,7 hPa
	Temperatura wody:	(21,20 ÷ 21,25) ± 0,20 °C
DATA WZORCOWANIA	18 lutego 2022 r.	
SPÓJNOŚĆ POMIAROWA	Świadectwo jest wydane w ramach porozumienia EA MLA w zakresie wzorcowania i potwierdza spójność pomiarową wyników pomiarów z jednostkami miar Międzynarodowego Układu Jednostek Miar (SI).	
WYNIKI WZORCOWANIA	Podano na stronie 2 niniejszego świadectwa wraz z wartościami niepewności pomiaru.	
NIEPEWNOŚĆ POMIARU	Niepewność pomiaru została określona zgodnie z dokumentem EA-4/02 M:2021. Podane wartości niepewności stanowią niepewności rozszerzone przy prawdopodobieństwie rozszerzenia około 95 % i współczynniku rozszerzenia k = 2.	

KIEROWNIK
Laboratorium Pomiarowego
Tomasz Jędrzejewski

Niniejsze świadectwo może być okazywane lub kopiowane tylko w całości.

Photo 27. Multi-channel pipette calibration certificate

RADWAG Wagi Elektroniczne Witold Lewandowski

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METROLOGY, RESEARCH AND CERTIFICATION CENTER – MEASURING LABORATORY

26-600 Radom, ul. Starowiejska 17A

tel. 48 386 64 70; fax. 48 385 00 11

The calibration laboratory accredited by Polish Center for Accreditation, a signatory of EA MLA and ILAC MRA agreements on mutual acknowledgment of the calibration certificates.

Accreditation number AP 069.

CALIBRATION CERTIFICATE

Date of issue: 18 February 2022 Certificate no.: 2136/470/22 Page: 1/2

CALIBRATED ITEM	Eight-channel piston pipette
	Type: adjustable-volume
	Manufacturer: Eppendorf
	Serial number: J38259G
	Volume: 10-100 µl
	Tip type: supplied by the ordering party
REPORTING PARTY	
USER	
CALIBRATION METHOD	Calibration procedure: PW 05 issue II as of 14 October 2021
ENVIRONMENTAL	
CONDITIONS	Air temperature: (21.73 ÷ 21.78) ± 0,20 ºC
	Relative air humidity: (48.8 ÷ 50.2) ± 1.2%
	Atmospheric pressure: (986.5 ÷ 995.1) ± 0.7 hPa
	Water temperature: (21.20 ÷ 21.25) ± 0.20 ºC
CALIBRATION DATE	18 February 2022
MEASUREMENT	
TRACEABILITY	The certificate is issued under EA MLA agreement with regard to calibration and confirms the measurement traceability of results with units of measure of the International System of Units (SI).
CALIBRATION RESULTS	Given in the page 2 of this certificate together with measurement uncertainty values.
MEASUREMENT	The measurement uncertainty was specified as per EA-4/02 M:2021
UNCERTAINTY	document. Uncertainty values serve as expanded uncertainties at
	expansion probability of about 95% and expansion coefficient k=2.

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This certificate can be presented or copied in full only.

Świadectwo wzorcowania wydane przez LABORATORIUM AKREDYTOWANE Nr AP 069

Data wydania: 18 lutego 2022 r. Nr świadectwa: 2136/470/22 Strona: 2 / 2

WYNIKI WZORCOWANIA Wyniki przeprowadzonego wzorcowania przedstawione poniżej, odnoszą się wyłącznie do obiektu wzorcowania opisanego na pierwszej stronie świadectwa.

Objętość nominalna V_0 µl	Wartość objętości zmierzona V µl	Wartość bł. systemat. es µl	Wartość bł. systemat. es %	Wartość bł. przypad. Sr µl	Wartość bł. przypad. CV %	Niepewność pomiaru U µl
100 µl Kanał						
1	100,05	0,05	0,05	0,15	0,15	0,27
2	100,16	0,16	0,16	0,16	0,16	0,27
3	99,90	-0,10	-0,10	0,22	0,22	0,27
4	99,99	-0,01	-0,01	0,13	0,13	0,27
5	99,96	-0,04	-0,04	0,10	0,10	0,27
6	99,99	-0,01	-0,01	0,13	0,13	0,27
7	100,06	0,06	0,06	0,11	0,11	0,27
8	99,98	-0,02	-0,02	0,12	0,12	0,27
50 µl Kanał						
1	49,79	-0,21	-0,43	0,07	0,13	0,17
2	49,88	-0,12	-0,24	0,12	0,24	0,17
3	49,90	-0,10	-0,21	0,13	0,25	0,17
4	49,86	-0,14	-0,28	0,08	0,16	0,17
5	49,85	-0,15	-0,30	0,07	0,14	0,17
6	49,90	-0,10	-0,21	0,06	0,12	0,17
7	49,80	-0,20	-0,41	0,08	0,15	0,17
8	49,80	-0,20	-0,40	0,05	0,10	0,17
10 µl Kanał						
1	9,976	-0,024	-0,239	0,041	0,414	0,050
2	9,979	-0,021	-0,211	0,063	0,631	0,050
3	9,987	-0,013	-0,129	0,049	0,493	0,050
4	9,986	-0,014	-0,142	0,048	0,482	0,050
5	9,979	-0,021	-0,214	0,044	0,439	0,050
6	9,965	-0,035	-0,347	0,021	0,208	0,050
7	9,971	-0,029	-0,289	0,045	0,452	0,050
8	9,961	-0,039	-0,391	0,059	0,593	0,050

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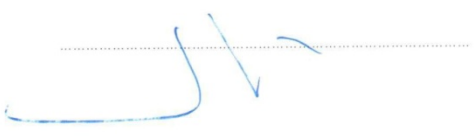

Photo 28. Multi-channel pipette calibration certificate – results

CALIBRATION RESULTS Results of the calibration, showed below, refer only to the calibrated item described in the first page of this certificate.

Nominal volume V_0 µl	Measured volume value V µl	Systematic error value e_s µl	Systematic error value e_s %	Random error value S_r µl	Random error value CV %	Measurement uncertainty U µl
100µl						
Channel						
1	100.05	0.05	0.05	0.15	0.15	0.27
2	100.16	0.16	0.16	0.16	0.16	0.27
3	99.90	-0.10	-0.10	0.22	0.22	0.27
4	99.99	-0.01	-0.01	0.13	0.13	0.27
5	99.96	-0.04	-0.04	0.10	0.10	0.27
6	99.99	-0.01	-0.01	0.13	0.13	0.27
7	100.06	0.06	0.06	0.11	0.11	0.27
8	99.98	-0.02	-0.02	0.12	0.12	0.27
50µl						
Channel						
1	49.79	-0.21	-0.43	0.07	0.13	0.17
2	49.88	-0.12	-0.24	0.12	0.24	0.17
3	49.90	-0.10	-0.21	0.13	0.25	0.17
4	49.86	-0.14	-0.28	0.08	0.16	0.17
5	49.85	-0.15	-0.30	0.07	0.14	0.17
6	49.90	-0.10	-0.21	0.06	0.12	0.17
7	49.80	-0.20	-0.41	0.08	0.15	0.17
8	49.80	-0.20	-0.40	0.05	0.10	0.17
10µl						
Channel						
1	9.976	-0.024	-0.239	0.041	0.414	0.050
2	9.979	-0.021	-0.211	0.063	0.631	0.050
3	9.987	-0.013	-0.129	0.049	0.493	0.050

4	9.986	-0.014	-0.142	0.048	0.482	0.050
5	9.979	-0.021	-0.214	0.044	0.439	0.050
6	9.965	-0.035	-0.347	0.021	0.209	0.050
7	9.971	-0.029	-0.289	0.045	0.452	0.050
8	9.961	-0.039	-0.391	0.059	0.593	0.050

Authorized by: Tomasz Jędrzejewski

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8. Attachment 1

8.1. Physical phenomena and measuring principles during piston pipette control

Evaluation of all factors that may potentially influence accuracy of collecting liquid through a piston pipette with an airbag requires presentation of this process in a mathematical form. A similar process is isothermal change of the state of matter of the perfect gas that can be described through the equation (1).

$$P_a \times V_o = \left(P_a - P_h - P_s\right) \times \left(V_O - V_{str} - V\right) \quad (1)$$

where: P_a *air pressure*
P_h *hydrostatic pressure*
P_s *capillary pressure*
V_o *entrapped air volume*
V_{str} *piston stroke volume*
V *sucked liquid volume*

The value of the hydrostatic pressure triggered by the column of liquid (in the pipette tip) depends on several factors included in the equation (2).

$$P_h = \rho_w \times g \times h \quad (2)$$

where: ρ_w *liquid (water) density*
g *gravitational acceleration*
h *altitude of column of liquid inside the tip*

Considering dependencies presented in the equation (1) and (2), the volume of the sucked liquid *) with regard to effects related to liquid evaporation, temperature differences and pipette use can be calculated as per the equation (3)

$$V = V_{str} - V_o \times \frac{\left(\rho \times g \times h\right) + P_s}{P_a - \left(\rho \times g \times h\right) - P_s} - V_{ev} \pm V_{Tdiff} \pm V_{handling} \quad (3)$$

where:

V_{ev} *drop of volume as a result of liquid evaporation to airbag during suction*
V_{Tdiff} *impact of the temperature change in the airbag during suction*
$V_{handling}$ *change of volume caused by various use effects*

The main elements and stages of pipetting have been showed in the photo 29. Before pipetting starts, the pipette piston (1) is in the starting position (5), empty space (2) contains the so-called airbag. The pipette tip (4) is slightly immersed in liquid. The structure tightness is assured by a pipette casing (3).

After collecting the liquid, the piston moves to the final position (6), thus expanding the air trapped in the airbag, the liquid fills the tip in to (h) level. At this moment liquid evaporates to the airbag, which leads to increase in its volume. Increased volume of the airbag in turn causes a small amount of liquid to be pushed out of the pipette tip. In effect the volume of the dose of liquid is lower. Temperature changes ΔT/T that may occur during this process change the volume of the airbag V_{eff}, which also results in changes to volume of the liquid supplied.

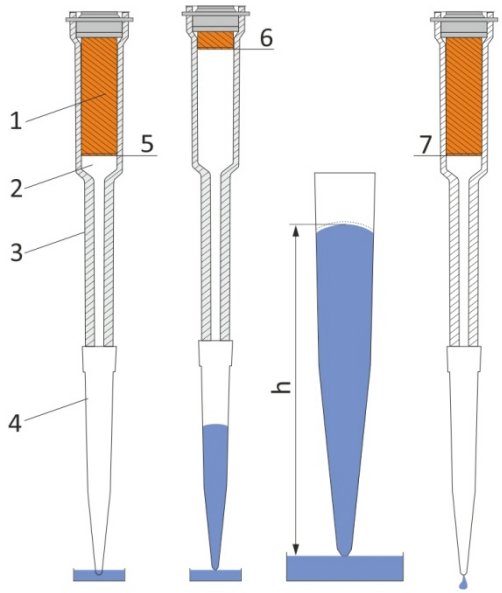

Photo 29. Piston pipette - liquid collection stages

While expelling the liquid, the piston moves to the position (7) and liquid is removed from the pipette tip. Aside from physical factors, an essential influence on pipetting accuracy may be exerted also by human factor. In this case the following factors can be distinguished:

- inclination angle while collecting liquid
- waiting time after aspiration,
- time during which the tip is still immersed in test liquid,
- time between further aspirations,
- tip immersion depth (in test liquid),
- working force affecting the piston,
- heat transfer from operator's hand into pipette structure

The equation 3 describes physical aspects related to heat transfer, such as evaporation and temperature fluctuations. A detailed analysis of this phenomenon can be conducted with the use of equation 4, where other pipette-related factors have been taken into account.

$$V = V_{str} - V_o \times \frac{(\rho \times g \times h) + P_s}{P_a - (\rho \times g \times h) - P_s} + \left[A_{fl} \times P_d \times (1 - S) \times k \times t_{AnsW}\right] \pm \left[V \times m \times \frac{\Delta T}{T}\right] - + \left[A_{fl} \times b \times \frac{\Delta T}{T}\right] \pm$$

(4)

where A_{fl} *contact surface between liquid and airbag*
P_d *liquid steam pressure*
S *airbag steam saturation in % RH*
t_{AnsW} *aspiration time plus waiting time*
k *evaporation coefficient (volume per surface, steam pressure and time)*
V *suction volume*
M *coefficient providing for radial heat conduction. m = 0,3 …. 0,9, depending on pipette size*
b *coefficient providing for axial heat conduction (volume per surface, time dependent upon* t_{AnsW}*).*
$\Delta T/T$ *relative temperature change in airbag*

Values of some coefficients, such as S, k, m, are not known and must be determined experimentally, which is a challenge, mainly in the research stand area.

*) source: Feldmann, R., Lochner, K.H. Influences on volume in piston-operated air-displacement pipettes. Accred Qual Assur 21, 69–82 (2016). https://doi.org/10.1007/s00769-015-1171-y

8.2. Maximum permissible errors of piston pipettes

Table 5. Maximum permissible error levels for A- and D1-type pipettes (single-channel)

Pipetting volume		**Max. permissible systematic error [a]**	**Max. permissible random error [a]**
Rated volumes µl	Value as rated volume proportion (%)	± %	% b
1 to 3[c]	100	2,5	2,0
	50	5,0	4,0
	10	25	20
> 3 to 5	100	2,5	1,5
	50	5,0	3,0
	10	25	15
> 5 to 10	100	1,2	0,8
	50	2,4	1,6
	10	12	8,0
> 10 to 50	100	1,0	0,5
	50	2,0	1,0
	10	10	5,0
> 50 to 5 000	100	0,80	0,30
	50	1,6	0,60
	10	8,0	3,0
> 5 000 to 20 000	100	0,60	0,30
	50	1,2	0,60
	10	6,0	3,0

[a] – to calculate errors in micro liters, multiply values of max. permissible errors by the specific volume.

[b] – expressed as a variability coefficient as per ISO 8655-6, ISO 8655-7 or ISO 8655-8 standards.

[c] – processing small amounts can be very difficult.

Table 6. Maximum permissible error levels for A- and D1--type pipettes (multi-channel)

Pipetting volume		**Max. permissible systematic error** [a]	**Max. permissible random error** [a]
Rated volumes µl	Value as rated volume proportion (%)	± %	% [b]
2[c]	100	8,0	8,0
	50	16	16
	10	25	25
> 2 to 5	100	5,0	3,0
	50	10	6,0
	10	25	25
> 5 to 10	100	2,4	1,6
	50	4,8	3,2
	10	24	16
> 10 to 20	100	2,0	1,0
	50	4,0	2,0
	10	20	10
> 20 to 50	100	2,0	0,80
	50	4,0	1,6
	10	20	8,0
> 50 to 2 000	100	1,6	0,60
	50	3,2	1,2
	10	16	6,0

[a] – to calculate errors in micro liters, multiply values of max. permissible errors by the specific volume.

[b] – expressed as a variability coefficient as per ISO 8655-6, ISO 8655-7 or ISO 8655-8 standards.

[c] – processing small amounts can be very difficult.

Table 7. Maximum permissible error levels for D2-type pipettes

Pipetting volume		**Max. permissible systematic error [a]**	**Max. permissible random error [a]**
Rated volumes µl	Value as rated volume proportion (%)	± %	% [b]
5[c]	100	2,5	1,5
	50	5,0	3,0
	10	25	15
> 5 to 10	100	2,0	1,0
	50	4,0	2,0
	10	20	10
> 10 to 20	100	2,0	0,80
	50	4,0	1,6
	10	20	8,0
> 20 to 100	100	1,4	0,60
	50	2,8	1,2
	10	14	6,0
> 100 to 1 000	100	1,2	0,40
	50	2,4	0,80
	10	12	4,0

[a] – to calculate errors in micro liters, multiply values of max. permissible errors by the specific volume.

[b] – expressed as a variability coefficient as per ISO 8655-6, ISO 8655-7 or ISO 8655-8 standards.

[c] – processing small amounts can be very difficult.

8.3. Z correcting indicator values for distilled water

Table 8. Z correcting indicators for distilled water as a water temperature and air pressure function – Z values given in μl/mg

Temperature (°C)	**Air pressure** (Kpa)						
	80	**85**	**90**	**95**	**100**	**101,3**	**105**
15,0	1001 7	1001 8	1001 9	1001 9	1002 0	1002 0	1002 0
15,5	1001 8	1001 9	1001 9	1002 0	1002 0	1002 0	1002 1
16,0	1001 9	1002 0	1002 0	1002 1	1002 1	1002 1	1002 2
16,5	1002 0	1002 0	1002 1	1002 1	1002 2	1002 2	1002 2
17,0	1002 1	1002 1	1002 2	1002 2	1002 3	1002 3	1002 3
17,5	1002 2	1002 2	1002 3	1002 3	1002 4	1002 4	1002 4
18,0	1002 2	1002 3	1002 3	1002 4	1002 5	1002 5	1002 5
18,5	1002 3	1002 4	1002 4	1002 5	1002 5	1002 6	1002 6
19,0	1002 4	1002 5	1002 5	1002 6	1002 6	1002 7	1002 7
19,5	1002 5	1002 6	1002 6	1002 7	1002 7	1002 8	1002 8
20,0	1002 6	1002 7	1002 7	1002 8	1002 8	1002 9	1002 9
20,5	1002 7	1002 8	1002 8	1002 9	1002 9	1003 0	1003 0
21,0	1002 8	1002 9	1002 9	1003 0	1003 1	1003 1	1003 1
21,5	1003 0	1003 0	1003 1	1003 1	1003 2	1003 2	1003 2
22,0	1003 1	1003 1	1003 2	1003 2	1003 3	1003 3	1003 3
22,5	1003 2	1003 2	1003 3	1003 3	1003 4	1003 4	1003 4
23,0	1003 3	1003 3	1003 4	1003 4	1003 5	1003 5	1003 6
23,5	1003 4	1003 5	1003 5	1003 6	1003 6	1003 6	1003 7
24,0	1003 5	1003 6	1003 6	1003 7	1003 7	1003 8	1003 8
24,5	1003 7	1003 7	1003 8	1003 8	1003 9	1003 9	1003 9
25,0	1003 8	1003 8	1003 9	1003 9	1004 0	1004 0	1004 0
25,5	1003 9	1004 0	1004 0	1004 1	1004 1	1004 1	1004 2
26,0	1004 0	1004 1	1004 1	1004 2	1004 2	1004 3	1004 3
26,5	1004 2	1004 2	1004 3	1004 3	1004 4	1004 4	1004 4
27,0	1004 3	1004 4	1004 4	1004 5	1004 5	1004 5	1004 6
27,5	1004 5	1004 5	1004 6	1004 6	1004 7	1004 7	1004 7
28,0	1004 6	1004 6	1004 7	1004 7	1004 8	1004 8	1004 8
28,5	1004 7	1004 8	1004 8	1004 9	1004 9	1005 0	1005 0
29,0	1004 9	1004 9	1005 0	1005 0	1005 1	1005 1	1005 1
29,5	1005 0	1005 1	1005 1	1005 2	1005 2	1005 2	1005 3
30,0	1005 2	1005 2	1005 3	1005 3	1005 4	1005 4	1005 4

9. List of photos

10. List of tables

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