

PIPETTE POLE IDEAL PARS

STANDARD OPERATING PROCEDURE (SOP) FOR P.I.P. PIPETTES

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# Introduction

The Standard Operating Procedure for calibration is a document to verify P.I.P. pipette performance in laboratories. Optimal operation of pipettes is achieved through regular calibration and proper maintenance. Poor calibration may lead to a malfunction that can directly affect testing.

The calibration process should be done by laboratories accredited for compliance and authorized to issue calibration certificates for P.I.P. pipettes.

### Purpose

This document describes a method for pipette calibration, enabling users to calculate and compare measurement errors in accordance with ISO 8655 and adjust the pipette if necessary.

### Scope

This SOP relates to all procedures utilizing P.I.P. pipettes.

### Responsibilities

Accredited laboratories authorized to issue calibration certificates are responsible for the implementation of this Standard Operating Procedure.

### 1.Glossary

### Accuracy

Accuracy of the actual value compared to the set value. (Figure 1)

### Calibration

Measuring process to reliably and reproducibly determine and document the error of measurement of a pipette.

### Maximum Permissible Errors

Upper or lower permitted extreme value for the deviation of the dispensed volume from the nominal volume. The maximum permissible errors are specified in accordance with ISO 8655.

### Nominal Volume

The maximum dispensing volume of a pipette specified by the manufacturer. P.I.P. pipette nominal volume is noticeable on both push button and P.I.P. Certificate.

### Precision

The scattering range of the measured values around the set value. A small scattering range represents a high level of precision. A large scattering range represents a low level of precision. (Figure 1)

### Random Error/Imprecision

A measure for the scattering (standard deviation) of the measured values around the average value.

### Systematic Error/Inaccuracy

Deviation of the average value of the dispensed volumes from the selected volume.

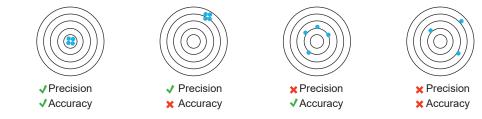


Figure1. Precision & Accuracy

# 2.Safety

If the pipette is contaminated, clean and decontaminate the device in accordance with the decontamination process before transport.

# Caution! contact with a contaminated device may result in personnel severe diseases.

Refer to the Operating Manual for decontamination instructions.

# 3.Test Intervals

The change of the systematic and random error is a gradual process. It is especially accelerated by aggressive chemicals, frequency of use and improper handling.

ISO 8655 requires annual calibration for optimal performance of the pipette.

# 4.Test Types

There are different methods for testing a pipette performance:

### 4-1. Visual Inspection of Pipette

The easiest and most frequently performed check is a visual inspection for damage to and contamination of the pipette. To do this:

- Inspect the tip cone for scratches or cracks.
- Inspect the pipette for broken parts.
- Inspect the pipette for external contamination.
- Check if the piston runs freely.

### 4-2. Checking Leak Tightness of Pipette

To check if the pipette is leak-tight, perform a simple test as bellow:

### 4-2-1. Prerequisites

- Ambient temperature is constant
- Ambient temperature is between 20 -25 (°C)
- Relative humidity > 50 %
- Test liquid: Deionized water
- Pipette, test tip and test liquid are at ambient temperature

### 4-2-2. Procedure

1. Attach the pipette tip.

2. Fill and empty the pipette tip 5 times (pre-wetting). This saturates the vapor phase in the air cushion and no more test liquid evaporates.

3. Aspirate nominal volume.

4. Hang the pipette on a pipette stand in a vertical position.

The pipette can be held vertically with two fingers. The hand temperature must not be transferred to the pipette. 5. The pipetting system is leak-tight, if no liquid drop forms at the pipette tip within 15 seconds.

### 4-3. Intermediate Check/Quick-Check

The Quick-Check is a shortened calibration with 4 measurements per volume. With 4 measured values the statistical errors are not ensured. Therefore; the Quick-Check is no substitute for a complete calibration with 10 measured values per volume.

### 4-4. Gravimetric Test

In gravimetric test, a calibration with 10 measured values per volume is performed in controlled conditions. The user can freely determine the thresholds within the ISO 8655 thresholds.

### 5.Prerequisites for Gravimetric Test

To avoid distortion of the measuring results, errors caused by test equipment and test method must be minimized.

### 5-1. Test Equipment

### 1. Test Liquid

Distilled or deionized water, conforming grade 3 as specified in ISO 3696, is used as test liquid. The test liquid must meet the conductivity of  $\leq$  0.5 ms/m at 25 °C. (ISO 3696)

#### The water shall be at room temperature.

### 2. The Test Liquid Container

The container for the test liquid should be sealable with a lid. This protects the test liquid from contamination. In addition, it should meet sufficient capacity for all the test liquid likely to be required for the complete series of tests.

### 3. Analytical Balance

Appropriate balance should be used in the gravimetric test. The balance should be serviced, calibrated and certified by qualified technicians.

Minimum requirements for the balance are mentioned in table 1.

#### Table 1. Requirements for the analytical balances

Selected Volume	Resolution (mg)	Repeatability and Linearity (mg)	Standard Uncertainty of Measurement (mg)
1 - 10 µl	0.001	0.002	0.002
10 - 100 µl	0.010	0.020	0.020
100 - 1000 µl	0.100	0.200	0.200
1 - 10 ml	0.100	0.200	0.200
10 - 200 ml	1.000	2.000	2.000

If the standard uncertainty of measurement of the balance is known (e.g. from the balance calibration certificate), this may be used instead of the repeatability and linearity.

The standard uncertainty of measurement shall not be more than two to three times the resolution.

#### 4. Weighing Vessel

Weighing vessels are used for weighing samples. The weighing vessel in the gravimetric test should meet the following requirements:

- Size suitable for test volume
- Ratio of height to diameter about at least 3:1
- Equipped with lids (To minimize evaporation loss, especially when the volumes to be tested are less than 50 µl)



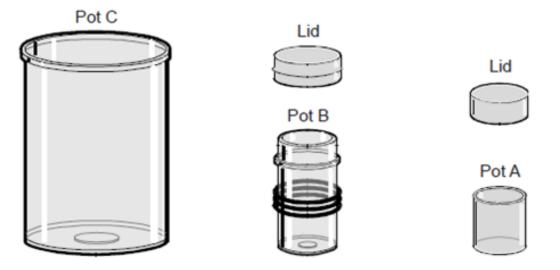


Figure 2. Weighing vessels

**Pot A container and lid:** For volumes up to 20 μl. It must be manipulated using tweezers to avoid hand warming. **Pot B container and lid:** For volumes from 20 to 200 μl. This container is fitted with O-rings to avoid hand warming. **Pot C container:** For volumes more than 200 μl.

### 5. Special Accessories to Minimize Evaporation

Especially for small volumes, errors due to evaporation of the test liquid during weighing shall be taken into consideration. In order to keep the error due to evaporation as small as possible, a balance with appropriate accessories such as an evaporation trap could be used.

### 6. Thermometer

A calibrated thermometer is used to measure the water and ambient temperature at the beginning and at the end of each test series. Use a thermometer with a maximum uncertainty of measurement of 0.2 °C.

### 7. Hygrometer

The hygrometer being used in the test shall have a standard maximum uncertainty of 10%.

### 8. Barometer

A standard uncertainty of less than or equal to 0.5 KPa should be considered for the barometer used in gravimetric test.

### 9. Timing Device

A timing device with a standard uncertainty of less than or equal to 1s shall be used.

### 5-2. Test Room

The test room should meet the following requirements:

- Draught-free with a stable environment
- Vibration-free Workplace
- Relative Humidity > 50 %
- Ambient Temperature 15 30 °C (±0,5 °C)
- No Direct Heat Radiation

### 5-3. Test Cycle

The duration of the test cycle must be as short as possible. ISO 8655 specifies a maximum time of 60 seconds.

### All influencing factors must be documented. (See Appendix D)

### 5-4. Procedure

### 5-4-1. General

• Test volume: In the case of P.I.P. pipettes, the test volume is the nominal volume.

• Number of measurements per test volume: If the gravimetric method is used as conformity tests, 10 measurements for each test volume shall be carried out.

# These measurements are used to calculate the systematic and random errors in accordance with clause 6.

### 5-4-2. Procedure Summary

1. Place test liquid from the water container in the weighing vessel to a depth of at least 3 mm.

2. Record the temperature of the test liquid, the barometric pressure and relative humidity in the test room.

# Temperature and barometric pressure are necessary for the choice of the correction factor Z. (See Appendix B)

3. If the weighing vessel has a lid, fit it.

4. Fit the selected tip to the pipette.

5. Fill the tip with test liquid and expel to waste (pre-wetting), five times to reach a humidity equilibrium in the dead air volume.

### Hold the pipette vertically to aspirate.

- 6. Place the weighing vessel with its added water on the balance pan.
- 7. Replace the disposable tip of the pipette.
- 8. Immerse the tip to the appropriate depth and pre-wet once.

### Press and release the operating button slowly.

- 9. Fill the pipette with test liquid.
- 10. Determine tare mass (reset balance).
- 11. Start the timing device. This may be omitted if using a weighing vessel with lid.

12. If the weighing vessel has a lid, remove it. Deliver the contents of the pipette into the weighing vessel.

Dispense the liquid touching the end of the pipette tip against the inside wall of the vessel at an angle of approximately 30° to 45°.

Press the push button to the second stop to expel the last drop of liquid.

If it is necessary to remove the weighing vessel from the balance pan to permit delivery of the dispensed volume, avoid excessive handling and possible contamination by the use of lint-free gloves.

13. After allowing the display to stabilize, record the mass.

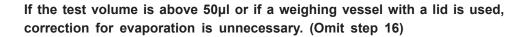
14. Repeat the test cycle (step 7-13) until ten measurements have been recorded.

15. Note the time to the nearest second taken to complete the 10 test cycles.

16. For samples below or equal to 50µl, estimate evaporation loss. After the last weighing, leave the weighing vessel on the balance pan for the time measured in step 15 and record its mass.

If the weighing vessel was removed from the balance pan to enable delivery, leave it on the pan for half the time of the step 15 and then remove it from the balance and allow it to stand on the workbench for half the time measured in step 15.

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17. Measure the temperature of the remaining test liquid, the barometric pressure, temperature and relative humidity in the test room again.

The calibration results must be documented. (See Appendix C)

### 6.Evaluation

6-1. Calculation of the Mean Mass

$$\overline{m} = \frac{1}{n} \sum_{i=1}^{n} m_i$$

m: Mean Mass
 n: Number of Measurements
 m<sub>i</sub>: Weighing Results

### 6-2. Conversion of the Mean Mass to Mean Volume

Where a mass loss has been determined to enable a correction for evaporation of the test liquid during the test cycle, calculate the mass loss per cycle.

 $\overline{V} = (\overline{m} + \overline{e}) \times Z$ 

 V
 Mean Volume

 m
 Mean Mass

 e
 Mass Loss (Evaporation Loss)

 Z: Correction Factor

E : Systematic Error  $V_0$ : Nominal Volume  $\overline{V}$ : Mean Volume

### 6-3. Calculating the Systematic Error

The systematic error is the measure of the deviation of the mean volume value from the target value.

### Absolute Systematic Error

Subtract the set nominal volume from the mean volume value.

$$\mathbf{E} = \overline{\mathbf{V}} - \mathbf{V}_0$$

### **Relative Systematic Error**

Multiply the absolute error by 100 and divide it by the nominal volume.

$$E\% = \frac{|\overline{v} - v_0|}{v_0} \times 100$$

### 6-4. Calculating the Random Error

The random error is a measure of the dispersion of the individual values around the mean volume value.

### Absolute Random Error

Calculate the standard deviation of the volume value.

 $\sigma = \sqrt{\frac{\sum_{i=1}^{n} (V_i - \overline{V})^2}{n-1}}$ 

- $\sigma$  : Standard Deviation
- $\overline{\mathrm{V}}$  : Mean Volume of the Measurements
- n : Number of Measurements
- V<sub>i</sub> : Individual Volumes:

### $V_i = m_i \times Z$

 $V_i$ : Individual Volume  $m_i$ : Weighing Results Z: Correction Factor

### **Relative Random Error**

Multiply the absolute error of measurement by 100 and divide it by the mean volume value.

$$CV = \frac{0}{\overline{V}} \times 100$$

CV: Coefficient of Variation

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For an example of how to evaluate the performance of P.I.P. pipette see appendix A.

### 6-5. P.I.P. Pipette Maximum Permissible Errors

To compare your measurements with P.I.P. errors of measurement, refer to table 2.

Table 2. P.I.P. pipette errors

PRODUCT No.	VOLUME	P.I.P. PIPETTE SYSTEMATIC ERROR	P.I.P. PIPETTE RANDOM ERROR	APPROPRIATE TIPS	MAXIMUM PERMISSIBLE	MAXIMUM
120260	• 1 µl	0.02 µl	0.01 µl	0.5-10 µl	O.05 μl	RANDOM ERROR 0.05 μl
120200	2 µl	0.02 µl	0.02 µl	0.5-10 µl	0.08 µl	0.04 µl
120201	3 µl	0.04 µl	0.03 µl	0.5-10 µl	0.125 µl	0.075 µl
120202	4 μl	0.05 µl	0.04 µl	0.5-10 µl	0.125 µl	0.075 µl
120203	5 μl	0.06 µl	0.04 µl	0.5-10 µl	0.12 µl	0.075 µl
120265	6 μl	0.07 µl	0.04 µl	0.5-10 µl	0.12µl	0.08 µl
120266	7 μl	0.08 µl	0.05 µl	0.5-10 µl	0.12 µl	0.08 µl
120200	8 µl	0.08 µl	0.05 µl	0.5-10 µl	0.12 µl	0.08 µl
120268	9 µl	0.09 µl	0.05 µl	0.5-10 µl	0.12 µl	0.08 µl
120298	10 µl	0.10 µl	0.05 µl	0.5-10 µl	0.12µl	0.08 µl
120269	• 10 µl	0.12 µl	0.08 µl	10-100 µl	0.2 µl	0.1 µl
120270	• 15 µl	0.18 µl	0.09 µl	10-100 µl	0.2 µl	0.1 µl
120271	• 20 ul	0.20 µl	0.10 µl	10-100 µl	0.2 µl	0.1 µl
120272	<ul> <li>25 μl</li> </ul>	0.29 µl	0.11 µl	10-100 µl	0.5 µl	0.2 µl
120273	• 30 µl	0.35 µl	0.12 µl	10-100 µl	0.5 µl	0.2 µl
120274	<ul><li>40 μl</li></ul>	0.44 µl	0.14 µl	10-100 µl	0.5 µl	0.2 µl
120275	<ul> <li>50 μl</li> </ul>	0.50 µl	0.15 µl	10-100 µl	0.5 µl	0.2 µl
120276	- 60 μl	0.58 µl	0.16 µl	10-100 µl	0.8 µl	0.3 µl
120277	• 70 μl	0.64 µl	0.18 µl	10-100 µl	0.8 µl	0.3 µl
120278	• 75 μl	0.66 µl	0.18 µl	10-100 µl	0.8 µl	0.3 µl
120279	• 80 µl	0.68 µl	0.18 µl	10-100 µl	0.8 µl	0.3 µl
120280	• 90 μl	0.70 µl	0.19 µl	10-100 µl	0.8 µl	0.3 µl
120281	• 100 μl	0.70 µl	0.20 µl	10-100 µl	0.8 µl	0.3 µl
120299	• 100 µl	0.80 µl	0.30 µl	100-1000 µl	0.8 µl	0.3 µl
120282	• 110 µl	0.90 µl	0.30 µl	100-1000 µl	1.6 µl	0.6 µl
120283	•120 µl	1.00 µl	0.30 µl	100-1000 µl	1.6 µl	0.6 µl
120284	• 150 µl	1.20 µl	0.30 µl	100-1000 µl	1.6 µl	0.6 µl
120285	•200 µl	1.60 µl	0.40 µl	100-1000 µl	1.6 µl	0.6 µl
120286	●220 µl	1.80 µl	0.40 µl	100-1000 µl	4 µl	1.5 µl
120287	●250 µl	2.00 µl	0.40 µl	100-1000 µl	4 µl	1.5 µl
120288	● 300 µl	2.40 µl	0.50 µl	100-1000 µl	4 µl	1.5 µl
120289	●400 µl	3.20 µl	0.70 µl	100-1000 µl	4 µl	1.5 µl
120290	•450 µl	3.60 µl	0.80 µl	100-1000 µl	4 µl	1.5 µl
120291	● 500 µl	4.00 µl	0.80 µl	100-1000 µl		1.5 µl
120292	•600 µl	4.80 µl	1.10 µl	100-1000 µl		3 µl
120293	•700 µl		1.40 µl	100-1000 µl		3 µI
120294	•750 µl		1.50 µl	100-1000 µl		3 µl
120295	• 800 µl		1.70 µl	100-1000 µl	·	3 µl
120296	• 900 µl	7.20 µl	2.00 µl	100-1000 µl		3 µl
120297	• 1000 µl		2.00 µl	100-1000 µl	8 µI	3 µl
P.I.P. pipet	te desired	fixed volume				

P.I.P. pipette desired fixed volume

# 7. P.I.P. Pipette Adjustment

### 7-1. Adjusting in case of Deviating Calibration Results

If the calibration results of mechanical pipettes are outside of the permissible thresholds, an adjustment may be necessary.

### Pipette adjustment does not influence the random error.

All external influencing factors listed as following must be ruled out before a pipette is adjusted.

- The tip cone is OK.
- The pipette tip is compatible with the pipette.
- The pipetting system is leak-tight.
- Test liquid was aspirated and dispensed 5 times (pre-wetting).
- The test liquid, dispenser and ambient air have the same temperature.
- The test liquid meets the ISO 3696 requirements.
- All the proper pipetting advices have been considered.

# Refer to the Operating Manual provided with your P.I.P. pipette for full details of proper pipetting advices.

- No draught at weighing location.
- Evaluation of measuring results performed correctly.

After all these parameters had been checked, decide if an adjustment is required.

### 7-2. Adjusting in case of Deviating Conditions

The physical properties of liquids and the ambient conditions are significant influencing factors for pipettes. Mechanical pipettes can be adjusted to these conditions.

Changing the adjustment is useful in the following cases:

- Liquids whose physical properties (density, viscosity, surface tension, vapor pressure & ...) differ significantly from those of water.
- Changes in the atmospheric pressure due to the altitude at which the pipette is used.
- Pipette tips whose geometry differs significantly from standard tips.

### Adjustment Procedure

To adjust P.I.P. pipettes:

- 1. Simply remove the push button.
- 2. Take out the color-coded cap.
- 3. Use an Allen Wrench to unlock the adjusting screw and adjust the volume.
- 4. Put the color-coded cap on.
- 5. Tighten everything back.
- 6. Use a gravimetric test to evaluate the measuring errors.
- 7. If the results are still outside of the permissible thresholds, a readjustment is necessary. Repeat steps 1-6.

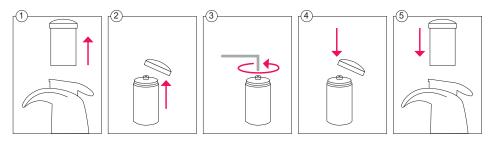
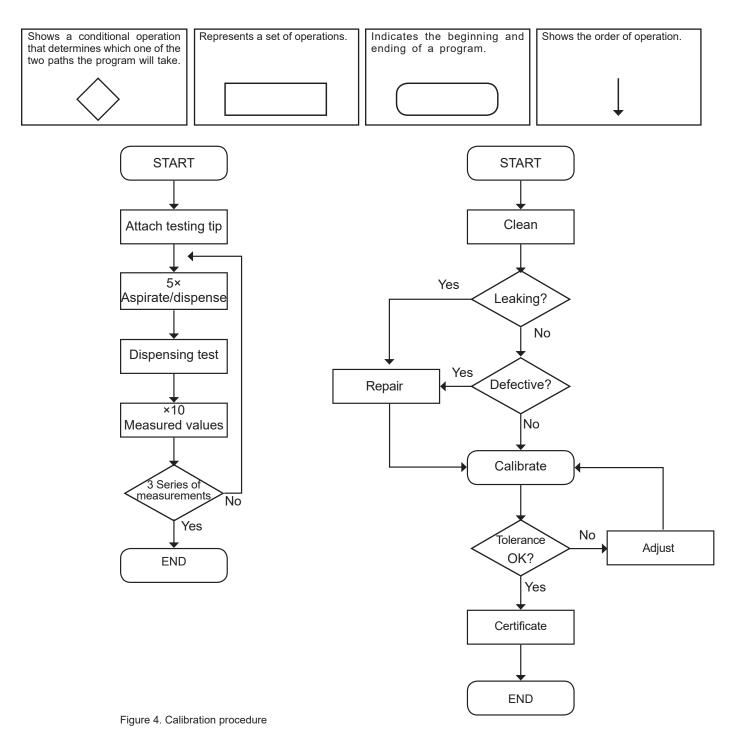


Figure 3. P.I.P. pipette adjustment

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# 8. Calibration Process Overview

The calibration involves various steps that are described in figure 4 (calibration diagram).



Appendix

### Appendix A: Example of a Performance Check

An example of how to evaluate the performance of P.I.P. pipette at 1 µl fixed volume:

**1.** Determine the mean value of the evaporation loss (ē) that occurs during your pipetting cycles. Here, it is 0.018 mg/per cycle.

2. Change the pipette tip. Then, keep a regular cycle and perform the 10 following measurements.

Calculate the Mean Mass:

$$\overline{m} = \frac{1}{n} \sum_{i=1}^{n} m_{i}$$

 $\overline{m}$  = (0.968 + 0.960 + 0.984 + 0.942 + 0.969 + 0.966 + 0.955 + 0.972 + 0.958 + 0.967) / 10  $\overline{m}$  = 0.964 mg

3. Calculate the mean volume for a temperature of 21.5°C and an air pressure of 101.3 KPa, the Z factor is equal to 1.0032  $\mu$ /mg (See Appendix B, table 3).

 $\overline{V} = (\overline{m} + \overline{e}) \times Z$  $\overline{V} = (0.964 + 0.018) \times 1.0032 = 0.985 \ \mu I$ 

4. Evaluate Accuracy / Systematic Error (E):

 $E = \overline{V} - V_0$ E = 0.985-1=0.015 µI

### 5. Relative Error (E%):

 $E\% = \frac{|\overline{v} - v_0|}{v_0} \times 100$ E\% = |0.015|/1 × 100=1.5%

### 6. Evaluate Precision / Random Error (σ):

$$\sigma_{\rm v} = \sqrt{\frac{\sum_{i=1}^{n} \left(V_i - \overline{V}\right)^2}{n-1}}$$

$$V_i = m_i \times Z$$

$$V_1 = 0.97 \ \mu l$$

$$V_2 = 0.96 \ \mu l$$

$$V_3 = 0.99 \ \mu l$$

$$V_4 = 0.95 \ \mu l$$

$$V_5 = 0.97 \ \mu l$$

$$V_6 = 0.97 \ \mu l$$

$$V_7 = 0.96 \ \mu l$$

$$V_8 = 0.98 \ \mu l$$

$$V_{10} = 0.97 \ \mu l$$

$$\overline{V} = \frac{1}{10} \sum_{i=10}^{n} V_i = 0.97$$

$$\left[ (0.97 - 0.97)^2 + (0.96 - 0.97)^2 \right]$$

$$\sigma_{v=1} \sqrt{\frac{(0.97 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.99 - 0.97)^{2} + (0.95 - 0.97)^{2} + (0.97 - 0.97)^{2} + (0.97 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.98 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97)^{2} + (0.96 - 0.97$$

 $\sigma_v\,{=}\,0.011\,\mu\text{I}$ 

### 7. Relative Error (CV):

$$CV = \frac{\sigma_v}{\overline{V}} \times 100$$
$$CV = 1.11\%$$

### Appendix B: Z Factor

Use the average of the first and the last values of temperature and barometric pressure to determine the correction needed (Z Factor).

The values of the conversion Z Factor ( $\mu$ I/mg) as a function of temperature and pressure for distilled water are shown in table 3.

Temperature				Air Pressure (KPa)			
(°C)	105	101.3	100	95	90	85	80
15.0	1.0020	1.0020	1.0020	1.0019	1.0019	1.0018	1.0017
15.5	1.0021	1.0021	1.0020	1.0020	1.0019	1.0019	1.0018
16.0	1.0022	1.0021	1.0021	1.0021	1.0020	1.0020	1.0019
16.5	1.0022	1.0022	1.0022	1.0021	1.0021	1.0020	1.0020
17.0	1.0023	1.0023	1.0023	1.0022	1.0022	1.0021	1.0021
17.5	1.0024	1.0024	1.0024	1.0023	1.0023	1.0022	1.0022
18.0	1.0025	1.0025	1.0025	1.0024	1.0023	1.0023	1.0022
18.5	1.0026	1.0026	1.0025	1.0025	1.0024	1.0024	1.0023
19.0	1.0027	1.0027	1.0026	1.0026	1.0025	1.0025	1.0024
19.5	1.0028	1.0028	1.0027	1.0027	1.0026	1.0026	1.0025
20.0	1.0029	1.0029	1.0028	1.0028	1.0027	1.0027	1.0026
20.5	1.0030	1.0030	1.0029	1.0029	1.0028	1.0028	1.0027
21.0	1.0031	1.0031	1.0031	1.0030	1.0029	1.0029	1.0028
21.5	1.0032	1.0032	1.0032	1.0031	1.0031	1.0030	1.0030
22.0	1.0033	1.0033	1.0033	1.0032	1.0032	1.0031	1.0031
22.5	1.0034	1.0034	1.0034	1.0033	1.0033	1.0032	1.0032
23.0	1.0036	1.0035	1.0035	1.0034	1.0034	1.0033	1.0033
23.5	1.0037	1.0036	1.0036	1.0036	1.0035	1.0035	1.0034
24.0	1.0038	1.0038	1.0037	1.0037	1.0036	1.0036	1.0035
24.5	1.0039	1.0039	1.0039	1.0038	1.0038	1.0037	1.0037
25.0	1.0040	1.0040	1.0040	1.0039	1.0039	1.0038	1.0038
25.5	1.0042	1.0041	1.0041	1.0041	1.0040	1.0040	1.0039
26.0	1.0043	1.0043	1.0042	1.0042	1.0041	1.0041	1.0040
26.5	1.0044	1.0044	1.0044	1.0043	1.0043	1.0042	1.0042
27.0	1.0046	1.0045	1.0045	1.0045	1.0044	1.0044	1.0043
27.5	1.0047	1.0047	1.0047	1.0046	1.0046	1.0045	1.0045
28.0	1.0048	1.0048	1.0048	1.0047	1.0047	1.0046	1.0046
28.5	1.0050	1.0050	1.0049	1.0049	1.0048	1.0048	1.0047
29.0	1.0051	1.0051	1.0051	1.0050	1.0050	1.0049	1.0049
29.5	1.0053	1.0052	1.0052	1.0052	1.0051	1.0051	1.0050
30.0	1.0054	1.0054	1.0054	1.0053	1.0053	1.0052	1.0052

### Table 3. Z values (µl/mg)

Appendix C: Checklists for the Calibration Report The calibration results and all influencing factors must be documented. The following chapters describe the contents of a test report.

Name	
Last name	
Department	

### Test Pipette

Manufacturer	
Nominal Volume	
Serial Number	

### Test tip

Manufacturer	
Designation	
Volume	

### Analytical Balance

Manufacturer	
Model	
Serial Number	
Last Calibration	

### Test Conditions:

r Temperature (°C)
r Pressure (KPa)
elative Humidity (٪)
st Liquid Temperature (°C)
Factor
vaporation Loss ( $\overline{e}$ )
alibration Date



### **Measurement Series**

	Measured Values	
Measuring Quantity	Actual Value	Comment
Average Volume $(\overline{ abla})$		
Systematic Error (µI)		
Random Error (µI)		

### Appendix D: Checklists for the Preparation of the Calibration

The following checklists can be used in preparation to ensure that all necessary equipment is available at the time of calibration. For this reason, the tables contain checkbox columns (Yes, No).

### Test conditions

No.	Description	Yes	No
1	Vibration-free weighing table is available.		
2	The measuring place is draught-free.		
3	Relative humidity is > 50 %.		
4	Ambient temperature is between 15 - 30 (°C).		
5	Documenting temperature, humidity and air pressure ara documented.		
6	Pipette, pipette tips, test liquid etc. have ambient temperature.		
7	Thermometer, with a standard uncertainty of <0.2 °C is available.		
8	Hygrometer, with a standard uncertainty of <10% is available.		
9	Barometer, with a standard uncertainty of < 0.5 KPa is available.		
10	Timing device, with a standard uncertainty of <1s is available.		

### Test liquid

No.	Description	Yes	No
1	Test liquid is available (according to ISO 3696).		
2	Test liquid has ambient temperature.		
3	The reservoir for the test liquid should meet sufficient capacity for all the test liquid likely to be required for the complete series of tests.		



No.	Description	Yes	No
1	Pipette has been cleaned.		
2	Defective parts have been replaced.		
3	The pipette tip has been attached correctly.		

### Analytical balance and weighing vessel

Pipette and pipette tips

No.	Description	Yes	No
1	The balance is calibrated or a valid calibration certificate is available.		
2	Sensitivity is set according to the test volume.		
3	Weighing vessel volume is sufficient for 10 liquid discharges of the nominal volume.		
4	The balance is aligned horizontally.		
5	Balance is switched on at least 2h before calibration.		

# **PIPETTE** Single Channel Fixed Volume Pipettes **POLE IDEAL PARS**



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