Operation and Maintenance of Micropipette

1 INTRODUCTION

The proper operation and maintenance of the micropipette is fundamental to solution and sample preparations. The methods and procedures discussed here pertain to most commonly used laboratory solution and sample preparations.

2 SCOPE AND APPLICATION

2.1 OVERVIEW

The Eppendorf Research pipettes, Eppendorf Research plus pipettes, and Finnpipette F1 pipettes are piston-stroke pipettes that operate according to the air-cushion principle.

3 REQUIRED TRAINING

4 EQUIPMENT AND MATERIALS

4.1 APPARATUS AND MATERIALS

4.1.1 Finnpipette F1 variable volume
4.1.2 Eppendorf Research plus variable volume
4.1.3 Eppendorf Reference variable volume
4.1.4 Analytical balance

5 SAFETY PRECAUTIONS

5.1 SAFETY PRECAUTIONS

5.1.1 Use gloves and safety goggles when handling solutions and samples.
5.1.2 Use the 0.5-5 mL and 1-10 mL pipette only with filter inserted.
5.1.3 Do not lay down the pipette when a filled pipette tip is attached.
5.1.4 When changing the volume, do not exceed the volume range of the pipette. Otherwise, the pipette may be damaged or affect pipetting performance. Do not set volumes outside the pipette's specified volume range. Using excessive force

to turn the knob or ring outside the range may jam the mechanism and eventually damage the pipette.

5.2 WASTE DISPOSAL

5.2.1 Any solution or water sample containing acid should be neutralized with sodium bicarbonate and then disposed of in the sink in Room 205.
5.2.2 Pipette tips are solely designed for disposable usage.

6 SOLUTIONS AND REAGENTS

7 STANDARD SOLUTIONS

8 PROTOCOL

8.1 OPERATION

8.1.1 Adjust the pipette to the needed volume. For Eppendorf Research plus pipettes, turn the volume adjustment ring as depicted in fig. 4.1 in Reference 4. To increase the delivery volume, turn the ring counterclockwise. To decrease the delivery volume, turn it clockwise. The height of the control button changes as the volume is adjusted. The numbers on the volume display are to be read from the top to the bottom. The decimal places are below the hyphen. For Finnpipette F1 pipettes, you will pull the knob on the top of the pipette to activate the volume setting. To increase the delivery volume, turn the knob counterclockwise. To decrease the delivery volume, turn it clockwise. Lock the volume by pushing the knob down. The numbers on the volume display are to be read from left to right. The decimal place is shown.
8.1.2 Attach pipette tip tightly. Pay attention to color code: the color of the control button indicates the color of the appropriate pipette tip or rack.
8.1.3 Push and release the control button slowly at all times, particularly when working with high viscosity liquids.
8.1.4 Press control button down to first stop (measuring stroke), see fig. 1(a) in Reference 2.
8.1.5 Hold pipette vertically and immerse tip approximately 4mm into the liquid.
8.1.6 Let control button glide back ***slowly***, see fig 1(b) in Reference 2. Maintain the immersion depth to ensure no air is aspirated. For large volumes: wait approximately 3 seconds before removing the pipette tip from the liquid.
8.1.7 Slide tip out of the liquid along the inside of the vessel.
8.1.8 Wipe off any droplets with a lint-free tissue. Ensure that no liquid is aspirated out of the tip.
8.1.9 To dispense liquid, press control button down to second (final) stop (dispensing stroke).
8.1.10 To guarantee precision and accuracy, it is recommended that all new tips are

pre-wetted by aspirating and dispensing liquid two or three times before pipetting.

8.1.11 To eject the tip, point the pipette at a suitable waste receptacle and press the tip ejector with your thumb.

8.2 CALIBRATION

8.2.1 Micropipettes can be tested by weighing the volume of distilled deionized water pipetted onto a sufficiently sensitive analytical balance. The scale graduation value of the balance should be chosen according to the selected test volume of the pipette:

Volume Range	Under 10 µL	10-100 µL	Above 100 µL
Readable graduation	0.001 mg	0.01 mg	0.1 mg

8.2.2 The distilled deionized water, weighing vessel, pipette, and pipette tips must have reached the same temperature. This means that the pipette should be held in the calibration room for at least two hours before calibration to reach equilibrium with the test room conditions. Weighing should take place at 20-25° C (constant to ±0.5° C).

8.2.3 The pipette is checked with the maximum volume and with the minimum volume. Fill test liquid up to a height of 3 mm in the weighing vessel. The weighing vessel should be a clean 50 mL beaker. For piston-stroke pipettes, fill the pipette tip up 5x with test liquid and empty it (pre-wet), in order to create a moisture balance in the dead air volume. Replace the single-use tip, and pre-wet the tip 1x. In addition to follow proper operating procedure, rest the filled tip up against the wall of the weighing vessel at an angle. Dispense the test liquid slowly until the first stop (measuring stroke). Press the control button to the second stop (blow-out), and dispense the remaining liquid in the tip. Hold down the control button, and pull the tip up the vessel wall. Let the control button slide back into position. Do 10 repetitions with the minimum volume, and 10 with the maximum volume. Calculate the accuracy (A) and precision (cv) of both series.

8.2.4 Compare the results to the limits in Table 1 and 2. If the calculated results are within the selected limits, the adjustment of the pipette is correct. Also see Attachment 1 for spreadsheet and limits from the manufacturer.

TABLE 1. Maximum permissible errors for fixed volume pipettes according to ISO8655-2

Volume	Systematic Error		Random Error	
µL	±%	± µL[a]	±%[b]	± µL[c]
1	5.0	0.05	5.0	0.05
2	4.0	0.08	2.0	0.05
5	2.5	0.125	1.5	0.075
10	1.2	0.12	0.8	0.08
20	1.0	0.2	0.5	0.1
50	1.0	0.5	0.4	0.2
100	0.8	0.8	0.3[d]	0.3[d]
200	0.8	1.6	0.3[d]	0.6[d]
500	0.8	4.0	0.3	1.5

1000	0.8	8.0	0.3	3.0
2 mL	0.8	16	0.3	6.0
5 mL	0.8	40	0.3	15.0
10 mL	0.6	60	0.3	30.0

[a] Expressed as the deviation of the mean of a tenfold measurement from the nominal or selected volume.
[b] Expressed as the coefficient of variation of a tenfold measurement.
[c] Expressed as the repeatability standard deviation of a tenfold measurement.
[d] For piston pipettes of type D1 (positive displacement piston pipettes with reusable plunger and capillary) the maximum permissible errors may be ±0.4 %.

TABLE 2. Maximum permissible errors for variable volume pipettes according to ISO8655-2

Volume	Systematic Error		Random Error	
µL	±%	± µL[a]	±%[b]	± µL[c]
5	2.5	0.13	1.5	0.08
10	2.0	0.2	1.0	0.1
20	2.0	0.4	0.8	0.16
50	1.4	0.7	0.6	0.3
100	1.5	1.5	0.6	0.6
200	1.5	3.0	0.4	0.8
500	1.2	6.0	0.4	2.0
1000	1.2	12.0	0.4	4.0

[a] Expressed as the deviation of the mean of a tenfold measurement from the nominal or selected volume.
[b] Expressed as the coefficient of variation to a tenfold measurement.
[c] Expressed as the repeatability standard deviation of a tenfold measurement.

8.3 MAINTENANCE

8.3.1 Daily checking
The pipette should be checked at the beginning of each day for dust and dirt on the outside surfaces of the pipette. 70% ethanol should be used to clean the pipette.

8.3.2 Short-term service
If the pipette is used daily, it should be checked and lubricated every three months.

8.3.3 Long-term service
If the pipette is used daily, it should be checked and lubricated every six months.

8.3.4 Care and Maintenance
- Do not allow any liquid to enter the pipette.
- Do not clean the pipette with acetone or aggressive solutions.
- Use original spare parts and accessories (pipette tips) only.

8.3.5 Sterilization
The entire pipette can be sterilized by autoclaving it at 121° C (252° F) for a minimum of 20 minutes. After autoclaving, the pipette must be cooled to room temperature for at least two hours. Make sure that the pipette is dry before using. Checking the calibration after every sterilization cycle is recommended to achieve the best possible accuracy.

9 DATA REDUCTION AND STATISTICS

9.1 CALCULATIONS

9.1.1 Conversion of mass to volume

$$V = (w + e) * Z$$

V = volume (µL)
w = weight (mg)
e = evaporation loss (mg)
Z = conversion factor for mg/µL conversion

The factor Z is for converting the weight of the water to volume at test temperature and pressure. A typical value is 1.0032 µL/mg at 22° C and 95 kPa. More information is in Reference 1.

9.1.2 Accuracy

Accuracy is the difference between the dispensed volume and the selected volume of a pipette.

$$A = \bar{V} - V_0$$

A = Accuracy
V_0 = nominal volume
$\bar{V}$ = mean volume

9.1.3 Precision

Precision refers to the repeatability of the pipettings. It is expressed as the standard deviation(s) or coefficient of variation (cv).

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

S = standard deviation
$\bar{V}$ = mean volume
n = number of measurements

Standard deviation can be expressed as a relative value (cv).

$$CV = 100\% * \frac{S}{\bar{V}}$$

10 QUALITY ASSURANCE

11 ADDITIONAL INFORMATION

11.1 REFERENCES

11.1.1 Finpipette Focus Fixed Volume Instructions for Use
11.1.2 Eppendorf Unipipette 3190 Instruction Manual
11.1.3 Finpipette F1 Instructions for Use
11.1.4 Eppendorf Research plus Operating Manual
11.1.5 Eppendorf Standard Operating Procedure for Manual Dispensing Systems

11.2 ATTACHMENTS

11.2.1 EXCEL File: Pipette Monthly Calibration – Template

12 PREVIOUS ISSUES AND CHANGES

Document File Name	Issue	Issue Effective Dates	Author
Inst-Micropipette-001	001	April 1st, 2008 – April 12th, 2018	Jenny Xianghua Luo
07_02_11.002 Micropipette	002	April 12th, 2018 - Present	Victoria Wickham

12.1 ISSUE CHANGES

12.1.1 Issue 001:

- Original version

12.1.2 Issue 002:

- Moved SOP to new template
- Updated maximum permissible errors values for both fixed and variable volume pipettes according to ISO8655
- Added a table for maximum permissible errors according to ISO8655 so that both fixed and variable volumes were shown

13 DIAGRAMS, FIGURES, AND PHOTOGRAPHS