

Title: Calibration and Maintenance of Mechanical Pipettes

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Reference: Eppendorf Instruction Manual

<u>Rev. No.</u>	<u>Date Revised</u>	<u>Revision Summary</u>
1.	03-15-94	New SOP
2.	03-13-97	6.5 # of repeats
3.	08-08-99	Converted to LWP format. Sec. 6.5 added. Appendix revised.
4.	02-04-00	6.6.1: added
5.	10-24-01	Sec. 6.1: Changed to ambient temperature. Sec. 6.3: Added 500-2500 µL pipette. Sec. 6.8: Added statement for 500-2500 µL pipette. Appendix revised.
6.	12-27-02	Sec. 2.0: Wording change. Added 500-5000 µL pipette as sec. 6.3, 50-200 µL pipette as sec. 6.7, and renumbered accordingly. Sec.'s 6.3-6.5, 6.7: Changed to reflect lower limits. Sec.'s 4.1, 6.6: Removed 5-place balance. Sec. 6.8: Minor wording change. Sec. 6.10: Removed references to 500-2500 µL, 100-1000 µL, and 1-10 mL pipettes. Add sec.'s 6.11, 6.12: corrective action/investigation statement. Updated appendix.
7.	09-11-03	Sec.'s 6.3, 6.4, 6.5, 6.7, 6.8: Changed lower limits from 500 µL to 600 µL, 100 µL to 200 µL, 50 µL to 60 µL, and 1 mL to 2 mL. Sec. 6.5: Deleted 200-1000 µL pipette. Sec. 6.10: Added references to 500-2500 µL, 500-5000 µL, 100-1000 µL, 50-200 µL, and 1-10 mL pipettes. Updated Appendix.

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1.0 SCOPE AND APPLICATION

This SOP covers the calibration and maintenance of mechanical pipettes.

2.0 SUMMARY

Mechanical pipettes are to be calibrated, at the specified ranges outlined in section 6.0 of this SOP, on a weekly basis and documented in the appropriate log book. General maintenance is also described.

3.0 INTERFERENCES

Air drafts and temperature fluctuations should be eliminated prior to pipette calibration.

4.0 APPARATUS AND MATERIALS

4.1 Semi-micro balance, capable of weighing to within 0.0001 g.

4.2 Balance, capable of weighing to within 0.01 g.

4.3 Beaker and vial.

5.0 REAGENTS

5.1 Nanopure water at ambient temperature.

6.0 PROCEDURE

6.1 Calibration is carried out at ambient temperature.

6.2 Transfer water to a beaker and fill a vial with water.

6.3 500-5000 μL pipette: Rinse the purple pipette tip with 5000 μL of water. Pipette 5000 μL into a tared vial containing water on the 4-place balance. Document the weight. Set the pipette to 600 μL , pipette 600 μL into the vial and document the weight. See Appendix for a calibration summary form.

- 6.4 500-2500 μL pipette: Rinse the orange pipette tip with 2500 μL of water. Pipette 2500 μL into a tared vial containing water on the 4-place balance. Document the weight. Set the pipette to 600 μL , pipette 600 μL into the vial and document the weight. See Appendix for a calibration summary form.
- 6.5 100-1000 μL pipette: Rinse the blue pipette tip with 1000 μL of water. Pipette 1000 μL into a tared vial containing water on the 4-place balance. Document the weight. Set the pipette to 200 μL , pipette 200 μL into the vial and document the weight. See Appendix for a calibration summary form.
- 6.6 10-100 μL pipettes: Rinse the yellow pipette tip with 100 μL of water. Pipette 100 μL into a tared vial containing water on the 4-place balance. Document the weight. Set the pipette to 20 μL and pipette 20 μL into a vial filled to the rim with water and document the weight. See Appendix for a calibration summary form.
- 6.7 50-200 μL pipettes: Rinse the yellow pipette tip with 200 μL of water. Pipette 200 μL into a tared vial containing water on the 4-place balance. Document the weight. Set the pipette to 60 μL and pipette 60 μL into a vial filled to the rim with water and document the weight. See Appendix for a calibration summary form.
- 6.8 1-10 mL pipettes: Rinse the 10 mL pipette with 10 mL of water. Pipette 2 mL into a tared vial containing water on a 2-place balance. Document the weight. Set the pipette to 5 mL, pipette 5 mL into the vial and document the weight. Set the pipette to 10 mL, pipette 10 mL into the vial and document the weight. See Appendix for a calibration summary form.
- 6.9 For all pipettes, results should be $\pm 1\%$ at both the low and high ends. If you are confident of your technique and the result is outside of these limits, proceed with maintenance. If your technique is questionable, repeat the measurement until you are comfortable with the technique. Make sure the droplets do not stick to the outside of the tip. This is especially important at the low end of the 10-100 μL pipettes and can be avoided by dispensing more rapidly. Once you are comfortable with the technique proceed with one more measurement of the pipette. If the result is still out, refer to the maintenance section of this SOP as well as the troubleshooting section of the Eppendorf Instruction Manual.
- 6.9.1 Record all replicate measurements, flagging invalidated measurements and documenting the reason for the retest, following the principles of WCAS SOP #2230 (Retest of Out of Specification results). Use the back of the logbook page if necessary.
- 6.10 Calibration is not performed at 10 μL for the 10-100 μL pipettes, nor at 50 μL for the 50-200 μL pipettes, nor at 100 μL for the 100-1000 μL pipettes, nor at 500 μL for the 500-2500 μL and 500-5000 μL pipettes, nor at 1 mL for the 1-10 mL pipettes. Therefore, dispensing at these lower limits should be avoided.

- 6.11 If the pipette measurement remains outside the limits after the pipette has been cleaned and all applicable maintenance performed, remove the unit from service, document the removal in the log book, and send the pipette to an outside vendor for repair/calibration.
- 6.12 If the pipette is found to be out of tolerance at time of calibration, prior to any cleaning or maintenance, notify QA. QA will conduct an investigation and corrective action. Corrective action shall include:
 - 6.12.1 Coordinate the repair/calibration of the pipette with an outside vendor.
 - 6.12.2 Determine if a balance was used to verify the amount pipetted at time of use.
 - 6.12.3 Review all jobs, from the last acceptable calibration to time of deviation, that utilized the pipette in question and evaluate if the data was affected. If so, alert client(s) and make a note of all jobs/samples affected in case any re-analysis is required resulting from the deviation.

7.0 CLEANING AND MAINTENANCE

- 7.1 Digital pipettes do not require maintenance under normal use.
- 7.2 For the newer models, all parts of the pipette can be cleaned with a soap solution or isopropanol. After cleaning, rinse with distilled water and dry. The piston should then be lubricated lightly with silicone grease. See the trouble-shooting section of the manual if any of the following problems are encountered: droplets on the inside of the tip, dripping pipette or erratic movement of the control button. If replacement parts are needed, refer to the section of the manual detailing the replacement of these parts.
- 7.3 For the older models, if the nose cone gets clogged, it can be cleaned with distilled water as described in the older Eppendorf Instruction Manual. If the piston needs cleaning, it can be wiped with a damp cloth as also described in the Eppendorf manual. If cleaning the nose cone and/or piston does not solve pipetting problems, the pipette will probably have to be retired since Eppendorf no longer repairs or carries replacement parts for the older models.
- 7.4 Document all maintenance in the maintenance section of the pipette calibration logbook.

The following people have read this SOP and are currently using these procedures in the laboratory:

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APPENDIX
SAMPLE CALIBRATION LOG SHEETS

PIPETTE CALIBRATION

DATE: _____ **INITIALS:** _____

500-2500 uL pipettes

Serial #	Weight of 600 μ L water (g)	Weight of 2500 μ L water (g)

Acceptance Limits: 0.5940-0.6060 g 2.4750-2.5250 g

100-1000 uL pipettes

Serial #	Weight of 200 μ L water (g)	Weight of 1000 μ L water (g)

Acceptance Limits: 0.1980-0.2020 g 0.9900-1.0100 g

10-100 uL pipettes

Serial #	Weight of 20 uL water (g)	Weight of 100 uL water (g)

Acceptance Limits: 0.0198-0.0202 g 0.0990-0.1010 g

1-10 mL pipette

Serial #	Weight of 2 mL water (g)	Weight of 5 mL water (g)	Weight of 10 mL (g)
ICP-6 (F89626)			

Acceptance Limits: 1.98-2.02 g 4.95-5.05 g 9.90-10.1 g

500-5000 uL pipettes

Serial #	Weight of 600 μ L water (g)	Weight of 5000 μ L water (g)

Acceptance Limits: 0.5940-0.6060 g 4.9500-5.0500 g

Analytical Balance ID: _____ Top-Load Balance ID: _____

Pipette Type: Eppendorf 500 - 5000 µL

Serial No.:
WCAS Pipette ID:

Date In Service:
Date Removed From Service:

Date	Initial	Weight of 5000 µL water (grams)	Weight of 600 µL water (grams)	Comments:

Acceptance Limits: 4.9500 - 5.0500 g 0.5940 - 0.6060 g

Pipette Type: Eppendorf 50 - 200 µL

Serial No.:
WCAS Pipette ID:

Date In Service:
Date Removed From Service:

Date	Initial	Weight of 200 µL water (grams)	Weight of 60 µL water (grams)	Comments:

Acceptance Limits: 0.1980 - 0.2020 g 0.0594 - 0.0606 g

Pipette Type: Eppendorf 10 - 100 µL

Serial No.:
WCAS Pipette ID:

Date In Service:
Date Removed From Service:

Date	Initial	Weight of 100 µL water (grams)	Weight of 20 µL water (grams)	Comments:

Acceptance Limits: 0.0990 - 0.1010 g 0.0198 - 0.0202 g

Reviewed by: _____

Date: _____

Comments: