PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Auto Pipette Calibration	**Effective Date:**
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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 Pure 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 ±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 SOP 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.

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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.

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I. INTRODUCTION

A. Purpose

1. This procedure provides a method for testing the accuracy and precision of automatic micropipets (Eppendorf style).
2. The method is valid for both fixed and variable volume pipets which dispense volumes down to 10 μL.

B. Procedure Outline

1. The method consists of measuring the weight of repeated aliquots of pipetted deionized water.

II. REQUIRED EQUIPMENT AND SUPPLIES

A. Equipment

1. Balance accurate to ± 0.1 mg
2. 100-ml plastic beakers
3. Pipettes
4. Calibrated weight set, ASTM Class 2, 0.001 to 100 g

B. Supplies

1. Pipet tips
2. Liquid scintillation vials or small beakers

C. Chemicals

1. Deionized water

III. PROCEDURE

A. Documentation

1. Using a copy of the attached form titled “Fixed-Volume Automatic Pipet Calibration” or “Adjustable-Volume Automatic Pipet Calibration”, as applicable, write the date and pipet identification information in the appropriate blanks.
2. The “Calibration Due Date” shall be 3 months from the current date.
3. Maintain the completed data sheets in the same laboratory as the pipet.
4. After the next calibration, sheets should transferred to, and maintained in a file, in the Department of Health Physics office for a period of at least 3 years.
5. Upon completion of the calibration:
   a) Sign and date the data sheet
   b) Complete and affix a calibration sticker to the calibrated micropipet. The sticker should be similar to that in Figure 1 and must contain the following information:

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(1) Name of person performing the calibration
(2) Calibration Due Date
(3) Actual dispensed volume
(4) For adjustable-volume pipets, the dial reading corresponding to each volume should also be recorded.
(5) Accuracy (For adjustable-volume pipets, the accuracy should be listed for each volume calibrated).

B. Calibration check of balance

1. Record the balance and weight set identification information in the appropriate blanks.
2. With the weight pan empty, tare the balance.
3. Select at least four of the data points from the table that span the expected range of use. At each point:
   a) Place the appropriate weight on the weighting pan.
   b) Record the indicated weight in the column labeled 'Reading'.
   c) If the weight is within the desired tolerance, initial the column labeled 'Satisfactory'.
4. If any data point falls outside of the required tolerance, perform a balance calibration using the appropriate procedure.

C. Calibration of a Fixed-Volume Pipette

1. Place a small liquid scintillation vial or beaker capable of holding least 20 times the pipet volume on the balance weighting pan.
2. Tare the balance and record the weight on line 0 under Run 1.
3. Carefully pipet deionized water into the vial. Record the balance reading to the nearest 0.0001 g under the weight column for Run 1.
4. Repeat the step 3 fifteen times, adding each aliquot to the last.
5. Calculate the difference in weights between subsequent aliquots. Record these values in the Δ weight column.
6. Calculate and record the average, standard deviation (σ), and twice the standard deviation (2σ) of the Δ weight values converted to µL (multiple the weight in grams by 1000).
7. Identify any aliquots for which the pipetted volume is greater than 2σ from the average value.
8. Calculate and record the pipet accuracy based on the following formula

$$\textbf{Accuracy (\%)} = \frac{\textbf{Pipet Volume - Average Value}}{\textbf{Pipet Volume}} \times 100$$

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9. Calculate and record pipet precision based on the following formula:

$$\textbf{Precision (\%)} = \frac{\sigma}{\textbf{Average Volume}} \times 100$$

10. If any aliquot is greater than 2σ from the average volume, or if either the accuracy or precision significantly exceed the values listed in Table 1, repeat section D as Run 2.

D. Calibration of Adjustable-Volume Pipet

1. Adjustable pipets shall be calibrated at least three volumes. These volumes shall include the minimum and maximum volumes, as well as a mid-range or often used volume. (e.g., If a 500 - 2500 µl pipet is routinely used to dispense 2000 µl volumes, it must be calibrated at 500, 2000, and 2500 µl.)
2. For each volume to be calibrated at:
   a) Adjust the pipet to read the target volume
   b) Record the volume on the data sheet.
   c) Follow the calibration steps in section C of this procedure. In step C.10, use the tolerances in Table 2 for determination satisfactory accuracy and precision.

IV. UNCERTAINTIES

A. Calibration of balance and balance weights

B. Fit/tightness of pipet tip

C. Mechanical wear on the pipet tip

V. REFERENCES

A. HPD-97-LAB-310 Calibration and calibration checks of laboratory balances

B. Eppendorf pipet manuals

C. Shugar, G.J.; Dean, J.A. The chemist's ready reference handbook. New York: McGraw-Hill, 1989.

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Figure 1. Micropipet calibration label for a fixed-volume pipet.

Calibrator: _____
Cal. Due Date: _____
Volume: _____
Precision: _____

Figure 2. Micropipet calibration label for an adjustable-volume pipet.

Calibrator: _____
Cal. Due Date: _____
Setting/Volume: _____/_____
Precision: _____
Setting/Volume: _____/_____
Precision: _____
Setting/Volume: _____/_____
Precision: _____

Table 1. Tolerances of fixed-volume micropipets

Capacity µl	Accuracy %	Precision %	Capacity µl	Accuracy %	Precision %
10	±1.2	<0.5	200	±0.6	<0.2
25	±1.0	<0.3	250	±0.6	<0.2
50	±0.7	<0.3	500	±0.6	<0.2
75	±0.7	<0.3	750	±0.6	<0.2
100	±0.6	<0.2	1000	±0.6	<0.2
150	±0.6	<0.2	2500	±0.6	<0.2

Table 2. Tolerances of adjustable-volume micropipets

Capacity µl	Accuracy* %	Precision* %	Capacity µl	Accuracy* %	Precision* %
0.5-10	±5.0 to ±1.0	<2.8 to <1.0	50-250	±1.0 to ±0.6	<0.3 to <0.2
2-20	±6.0 to ±0.8	<5.0 to <0.3	100-1000	±1.6	<0.3
10-100	±2.0 to ±0.7	<0.5 to <0.2	500-2500	±1.0	<0.2

* When two values are listed, the first is for the minimum capacity, the second for the maximum.

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Fixed-Volume Automatic Pipet Calibration

Date: _____ Calibration Due Date: _____

Pipet Identification

Manufacturer: _____ Volume: _____ µL

Serial Number: _____ Laboratory Used In: BHS-_____

Calibration Check of Balance

Weight	Reading (g)	Tolerance	Satisfactory
20.0 g		±0.0002 g	
10.0 g		±0.0002 g	
5.0 g		±0.0002 g	
2.00 g		±0.0002 g	
0.100 g		±0.0001 g	
0.010 g		±0.0001 g	

Balance Manufacturer: _____

Balance Serial Number: _____

Laboratory: BHS-_____

Weight Set Serial Number: _____

Calibration of Pipette

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Run 1			Run 2		
	Weight (g)	Δ Weight (g)		Weight (g)	Δ Weight (g)
0			0		
1			1		
2			2		
3			3		
4			4		
5			5		
6			6		
7			7		
8			8		
9			9		
10			10		
11			11		
12			12		
13			13		
14			14		
15			15		
Average			Average		
1σ			1σ		
2σ			2σ		

Average: _____ µL
Accuracy: _____ %
Precision: _____ %

_____ _____

Analyst Date Laboratory Supervisor Date

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Adjustable-Volume Automatic Pipette Calibration

Date: _____ Calibration Due Date: _____

Pipet Identification

Manufacturer: _____ Volume Range: _____ - _____ µL

Serial Number: _____ Laboratory Used In: BHS-_____

Calibration Check of Balance

Weight	Reading (g)	Tolerance	Satisfactory
20.0 g		±0.0002 g	
10.0 g		±0.0002 g	
5.0 g		±0.0002 g	
2.00 g		±0.0002 g	
0.100 g		±0.0001 g	
0.010 g		±0.0001 g	

Balance Manufacturer: _____

Balance Serial Number: _____

Laboratory: BHS- _____

Weight Set Serial Number: _____

Calibration of Pipet

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Dial Setting:			**Dial Setting:**			**Dial Setting:**		
	Weight (g)	**Δ Weight (g)**		**Weight (g)**	**Δ Weight (g)**		**Weight (g)**	**Δ Weight (g)**
0			0			0		
1			1			1		
2			2			2		
3			3			3		
4			4			4		
5			5			5		
6			6			6		
7			7			7		
8			8			8		
9			9			9		
10			10			10		
11			11			11		
12			12			12		
13			13			13		
14			14			14		
15			15			15		
Average			**Average**			**Average**		
1σ			1σ			1σ		
2σ			2σ			2σ		
Accuracy			**Accuracy**			**Accuracy**		
Precision			**Precision**			**Precision**		

Analyst Date

Laboratory Supervisor Date