DISTRIBUTION: VistaLab Technologies Customers
EQUIPMENT: MLA Pipettes
SUBJECT: Pipette Performance Verification Protocol
PURPOSE: To evaluate the accuracy and precision performance of MLA Pipettes

I. INTRODUCTION

An experienced and properly trained technologist is required to accurately perform calibration of MLA Pipettes, using the gravimetric method. All procedures are to be performed under controlled environmental conditions (see Section III). Worksheets with example calculations are provided in Appendix A and Appendix B.

Some laboratories may not have access to facilities capable of performing the gravimetric method. In these instances they may wish to consider returning pipettes to VistaLab for repair and/or calibration; or, they may use other pipette verification procedures, such as the MLA Calibration Kit. This document addresses only the reference method: the gravimetric method.

II. MATERIALS REQUIRED FOR PERFORMANCE TESTING

A. Balance capable of weighing to a minimum of 4 decimal places (0.0001g)

- Balances should be regularly serviced and certified by a qualified technician, using weights trace-able to the National Institute of Standards and Technology (NIST). Between service calls, balances should be qualified using NIST-traceable weights; and they should be confirmed for stability, integration time, and levels.

- Balances should be stationed on marble tables mounted on elastomeric vibration isolator pads to minimize vibration.

B. Narrow mouthed weighing vessels, with a volume capacity of less than 50 times (preferably closer to 10 times) the test volume.
C. Calibrated thermometer (readable to 0.1ºC) to measure the temperature of the water.
D. MLA Pipette Tips of appropriate volume range must be used.
E. Hygrometer to determine humidity (optional for volumes of 100µL and greater).
F. Stopwatch
G. Non-aerated deionized or distilled water in an appropriate container.

III. ENVIRONMENT

A stringently controlled environment is necessary to ensure test reliability. Fluctuation in room temperature and humidity will adversely affect data.

Laboratory Test Room

Maintain the following laboratory conditions for at least two (2) hours prior to, and throughout, the evaluation procedure. Ensure that balances, water, pipettes, and tips are properly equilibrated to ambient conditions. Balances should be turned on at least one hour before use.

A. Temperature

Air Temperature 20 – 25 °C

Water Temperature 21 ± 1.0 °C

B. Relative Humidity

The calibration room should be maintained at 45–75% humidity; below 45% evaporation effects increase dramatically. (Humidity should be considered in the evaporation study.)

C. Barometric Pressure

The barometric pressure should be known to within ± 20 mm Hg, 25 mbar, 0.15 kPa, or 0.7 in. Hg.

D. Conditioning

Keep the room air circulating fan running continuously to prevent temperature surges. Drafts should be minimized to avoid drifts in measurement.

E. Lighting

Use diffused light of sufficient intensity. Avoid direct sunlight, which may cause a rise in temperature thereby affecting results.

IV. ESTIMATING THE EVAPORATION RATE

Evaporation is estimated by means of a series of simulated weighings to determine how much weight is lost because of evaporation during the weighing process.

The estimation is a two-step process. The first step determines how long a weighing process takes, and the second determines how much weight is lost during the elapsed time.

Measurement Timing Procedure

1. Fill an appropriate weighing vessel $^1/_4$ to $^1/_3$ full of water, and place it on the balance.
2. Perform a normal weighing cycle (see VI, 2–4), measuring the time from the moment the balance is tared until the balance settles after sample is added.
3. Repeat for a total of four measurements, and calculate the average time.

Evaporation Measurement Procedure

1. Perform a simulated weighing (see VI, 2–4); however, do not add water to the weighing vessel on the balance. Instead, return the distilled water that is still in the pipette tip to the reservoir from which it came.
2. Record the weight loss that occurs during the time interval measured above.
3. Repeat this procedure three (3) more times, and calculate the average weight loss.
4. Round the weight loss to the nearest 0.0001g, and convert it to a positive number. This is the evaporation rate *e*.
5. The evaporation rate *e* should be added to the mean measured mass when calculating the volume (see VII, D).

NOTE

Recalculate the evaporation rate every four hours or whenever ambient conditions change.

V. PIPETTE OPERATION

Pipetting consistency will significantly contribute to accuracy and reproducibility. Attention should be given to maintaining a steady rhythm when aspirating and dispensing samples; to speed and smoothness when pressing and releasing the plunger; and to tip immersion depth.

Pipettes should be held nearly vertical between the thumb and the middle finger with the plunger operated by the index finger (Figure 1). This holding method minimizes any hand-warming effects.

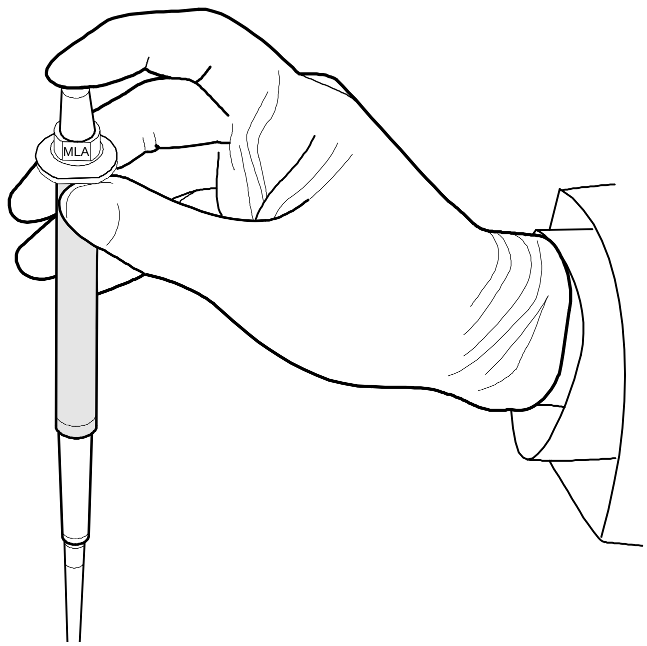

Figure 1. Proper Pipetting Technique

To Aspirate – for one-stroke pipettes

1. Press down the plunger completely, and immerse the pipette tip in the water according to Table 1.

Table 1. Pipette Immersion Depths

Volume	Immersion Depth
1–100µL	2–3mm
101–1000µL	2–4mm
1.1–10mL	3–6mm

2. Release the plunger with a uniform motion. Wait one second with the tip still immersed in the water. Withdraw the pipette tip smoothly by lifting straight up, either from the center of the water surface or up the side wall of the vessel.

NOTE

Do not permit further contact of the pipette or pipette tip with water once the liquid interface is broken.

3. Wipe the pipette tip only if there are extraneous droplets on the outside of the tip (use lint-free tissue only). Take care not to “wick” out any of the contents from the end of the pipette tip through contact with the orifice. Avoid flexing the pipette tip.

To Aspirate – for two-stroke pipettes

1. Press the plunger down to the first stop, and immerse the pipette tip in the water according to Table 1.
2. Release the plunger with a uniform motion. Wait one second with the tip still immersed in the water. Withdraw the pipette tip smoothly by lifting straight up, either from the center of the water surface or up the side wall of the vessel.

NOTE

Do not permit further contact of the pipette or pipette tip with water once the liquid interface is broken.

3. Wipe the pipette tip only if there are extraneous droplets on the outside of the tip (use lint-free tissue only). Take care not to “wick” out any of the contents from the end of the pipette tip through contact with the orifice. Avoid flexing the pipette tip.

To Dispense – for one-stroke pipettes

1. Place the pipette tip at an angle (10 to 45°) against the inside wall of the weighing vessel.
2. Fully press down the plunger in a uniform motion.
3. Slide the pipette tip up the inside wall of the weighing vessel to remove any drops that may have adhered to the outside or end of the tip.
4. Release the plunger with a uniform motion.

To Dispense – for two-stroke pipettes

1. Place the pipette tip at an angle (10 to 45°) against the inside wall of the weighing vessel.
2. Press the plunger slowly to the first stop. Pause.
3. Press the plunger farther to the second stop (blowout) or bottom of stroke; dispense all liquid from the tip.
4. Slide the pipette tip up the inside wall of the weighing vessel to remove any drops that may have adhered to the outside or end of the tip.
5. Release the plunger with a uniform motion.

VI. PROCEDURES FOR DETERMINING ACCURACY AND PRECISION

Accuracy of the pipette is determined by four (4) replicate cycles. Precision of the pipette is determined by ten (10) replicate cycles. To perform the accuracy or precision procedure, do the following:*

One-stroke Pipettes

1. Install a new, unused MLA Pipette Tip. Prior to the first cycle of a procedure, prerinse the tip with the same distilled water that is to be used for testing, following the guidelines in "Pipette Operation" (see V, "To Aspirate").
2. Place the appropriate weighing vessel on the balance and tare the balance with the door(s) closed.
3. When the balance reaches equilibrium, aspirate a sample of distilled water from the reservoir, and dispense it into the weighing vessel. Do not allow any time to elapse between aspiration and dispensing of liquid.
4. Close the balance door(s) and record the reading when the balance reaches equilibrium.
5. Repeat steps 3 and 4 above three (3) more times for accuracy, or nine (9) more times for precision.*

Two-stroke Pipettes

1. Place the appropriate weighing vessel on the balance and tare the balance with the door(s) closed.
2. Install a new, unused MLA PipetteTip. Do not prerinse the tip.
3. When the balance reaches equilibrium, aspirate a sample of distilled water from the reservoir, and dispense it into the weighing vessel. Do not allow any time to elapse between aspiration and dispensing of liquid.
4. Close the balance door(s) and record the reading when the balance reaches equilibrium.
5. Repeat steps 2 to 4 three (3) more times for accuracy, or nine (9) more times for precision.*

VII. TEST RESULTS

Record the results of each test including the test conditions. The data should include:

A. Measured test conditions

- Ambient air temperature (see III, A)
- Water temperature (see III, A)
- Humidity (see III, B)
- Barometric Pressure (see III, C)
- Z factor (see Table 2, Appendix A)

  (The Z factor is required in the volumetric calculations to compensate for the density of the water at the test conditions.)

B. The measured mean evaporation *e*

C. The 4 or 10 individual mass readings

D. The following values should be calculated:

- Mean Measured Mass

  Mean Measured Mass = (sum of individual weight measurements) / (number of readings)

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* During testing of an adjustable pipette, the first determination should be at the lowest volume setting, followed by testing at 60% and 100% of the maximum volume. For a selectable pipette, testing of all volumes is required.

- The corrected measured volume

  Measured Volume (corrected) = (Mean Mass + Mean Evaporation) $\times$ (Z factor from Table 2)
- % Accuracy (for precision and accuracy test)

$$\%\ Accuracy = \frac{(Measured\ \ Volume - Expected\ \ Volume)}{Expected\ \ Volume} \times 100$$

- Standard Deviation (for precision test)

$$SD = \sqrt{\frac{\sum M_i^2 - \frac{\left(\sum M_i\right)^2}{n}}{n-1}}$$

where:

M_i = individual weight measurement in grams

ΣM_i^2 = the sum of the squares of individual weight measurements

$(\Sigma M_i)^2$ = the square of the sum of individual weight measurements

n = 10

NOTE

The Standard Deviation formula given above is the algebraic equivalent of the more familiar:

$$SD = \sqrt{\frac{\sum_{i=1}^{n} (M_i - \overline{M})^2}{n-1}}$$

- % CV (for precision test)

$$\%\ CV = \frac{SD}{(\overline{M} + e_{average})} \times 100$$

where:

$e_{average}$ = evaporation average in grams

APPENDIX A

Z Factor Chart (mL/g)

To find the Z factor, locate the water temperature closest to the temperature measured during the test, then follow along that row to the column that represents the nearest Barometric Pressure measured during the test. That number is the Z factor (e.g., 18.0 ºC and 680 mm Hg = 1.0024 Z factor).

Table 2. Z Factor Chart

Barometric Pressure						
mm Hg	600	640	680	720	760	800
mbar	800	853	907	960	1013	1067
kPa	80.0	85.3	90.7	96.0	101.3	106.7
in. Hg	23.6	25.2	26.8	28.3	29.9	31.5
Water Temperature						
15.0	1.0018	1.0018	1.0019	1.0019	1.0020	1.0020
15.5	1.0018	1.0019	1.0019	1.0020	1.0020	1.0021
16.0	1.0019	1.0020	1.0020	1.0021	1.0021	1.0022
16.5	1.0020	1.0020	1.0021	1.0022	1.0022	1.0023
17.0	1.0021	1.0021	1.0022	1.0022	1.0023	1.0023
17.5	1.0022	1.0022	1.0023	1.0023	1.0024	1.0024
18.0	1.0022	1.0023	1.0024	1.0024	1.0025	1.0025
18.5	1.0023	1.0024	1.0025	1.0025	1.0026	1.0026
19.0	1.0024	1.0025	1.0025	1.0026	1.0027	1.0027
19.5	1.0025	1.0026	1.0026	1.0027	1.0028	1.0028
20.0	1.0026	1.0027	1.0027	1.0028	1.0029	1.0029
20.5	1.0027	1.0028	1.0028	1.0029	1.0030	1.0030
21.0	1.0028	1.0029	1.0030	1.0030	1.0031	1.0031
21.5	1.0030	1.0030	1.0031	1.0031	1.0032	1.0032
22.0	1.0031	1.0031	1.0032	1.0032	1.0033	1.0033
22.5	1.0032	1.0032	1.0033	1.0033	1.0034	1.0035
23.0	1.0033	1.0033	1.0034	1.0035	1.0035	1.0036
23.5	1.0034	1.0035	1.0035	1.0036	1.0036	1.0037
24.0	1.0035	1.0036	1.0036	1.0037	1.0038	1.0038
24.5	1.0037	1.0037	1.0038	1.0038	1.0039	1.0039
25.0	1.0038	1.0038	1.0039	1.0039	1.0040	1.0041
25.5	1.0039	1.0040	1.0040	1.0041	1.0041	1.0042
26.0	1.0040	1.0041	1.0042	1.0042	1.0043	1.0043
26.5	1.0042	1.0042	1.0043	1.0043	1.0044	1.0045
27.0	1.0043	1.0044	1.0044	1.0045	1.0045	1.0046
27.5	1.0044	1.0045	1.0046	1.0046	1.0047	1.0047
28.0	1.0046	1.0046	1.0047	1.0048	1.0048	1.0049
28.5	1.0047	1.0048	1.0048	1.0049	1.0050	1.0050
29.0	1.0049	1.0049	1.0050	1.0050	1.0051	1.0052
29.5	1.0050	1.0051	1.0051	1.0052	1.0052	1.0053
30.0	1.0052	1.0052	1.0053	1.0053	1.0054	1.0055

Source: *Determining Performance of Volumetric Equipment*, vol. 4 No. 6, National Committee for Clinical Laboratory Standards (NCCLS), May 1984

APPENDIX B

Pipette Accuracy Verification Worksheet (Sheet 1 of 1)

Date ______________________ **Technologist** ____

Test Conditions

Pipette ID _____________________________

Expected Volume ___________________ mL

Air Temperature __________________________ °C

Water Temperature ________________________ °C

Humidity ________________________________%

Barometric Pressure _________________________

Z factor (from Appendix A) __________________

Evaporation Measurement

Time		Evaporation	
t_1 _________________________	s	e_1 _________________________	g
t_2 _________________________	s	e_2 _________________________	g
t_3 _________________________	s	e_3 _________________________	g
t_4 _________________________	s	e_4 _________________________	g
$t_{average}$ ____________________	s	$e_{average}$ ____________________	g

Weight Measurements

Mass	
M_1 ________________________	g
M_2 ________________________	g
M_3 ________________________	g
M_4 ________________________	g
$M_{average}$ ____________________	g

Substitute your measured values in the two equations below.

Measured Volume (corrected) = ($M_{average}$ + $e_{average}$) × Z factor

________ mL = (________ + ________) × ________

% Accuracy = [(Measured Vol. – Expected Vol.) / Expected Vol.] × 100

________ % = [(________ – ________) / ________] × 100

Pipette Accuracy Verification Worksheet
Example Calculations

Date ____________________ **Technologist** ______________________________________

Test Conditions

Pipette ID ______________________________

Expected Volume ______*0.2*______ mL*

Air Temperature ______*24.0*______ °C

Water Temperature ______*22.0*______ °C

Humidity ______*61.0*______ %

Barometric Pressure ______*29.4*______

Z factor (from Appendix A) ___*1.0033*___

Evaporation Measurement

Time		Evaporation	
t_1	*18* s	e_1	*0.0038* g
t_2	*16* s	e_2	*0.0026* g
t_3	*17* s	e_3	*0.0022* g
t_4	*16* s	e_4	*0.0034* g
$t_{average}$	*17* s	$e_{average}$	*0.0030* g

Weight Measurements

	Mass
M_1	*0.19818* g
M_2	*0.19851* g
M_3	*0.19819* g
M_4	*0.19825* g
$M_{average}$	*0.19828* g

Substitute your measured values in the two equations below.

Measured Volume (corrected) = $(M_{average} + e_{average}) \times$ Z factor

_*0.2019*_ mL = (_*0.19828*_ + _*0.0030*_) × _*1.0033*_

% Accuracy = [(Measured Vol. – Expected Vol.) / Expected Vol.] × 100

__*+0.95*__ % = [(_*0.2019*_ – _*0.2000*_) / _*0.2000*_] × 100

* 1000 µL = 1 mL. To convert microliters to milliliters, divide microliters by 1000.

APPENDIX C

Pipette Precision Verification Worksheet (Sheet 1 of 2)

Date ______________________ **Technologist** ____

Test Conditions

Pipette ID ____________________________ Air Temperature _________________________ °C

Expected Volume ___________________ mL Water Temperature _______________________ °C

Humidity _________________________________%

Barometric Pressure ________________________

Z factor (from Appendix A) __________________

Evaporation Measurement

Time		Evaporation	
t_1 ________________________	s	e_1 ________________________	g
t_2 ________________________	s	e_2 ________________________	g
t_3 ________________________	s	e_3 ________________________	g
t_4 ________________________	s	e_4 ________________________	g
$t_{average}$ ____________________	s	$e_{average}$ ____________________	g

Weight Measurements

Mass		Mass^2	
M_1 ________________________	g	M_1^2 ________________________	g^2
M_2 ________________________	g	M_2^2 ________________________	g^2
M_3 ________________________	g	M_3^2 ________________________	g^2
M_4 ________________________	g	M_4^2 ________________________	g^2
M_5 ________________________	g	M_5^2 ________________________	g^2
M_6 ________________________	g	M_6^2 ________________________	g^2
M_7 ________________________	g	M_7^2 ________________________	g^2
M_8 ________________________	g	M_8^2 ________________________	g^2
M_9 ________________________	g	M_9^2 ________________________	g^2
M_{10} ________________________	g	M_{10}^2 ________________________	g^2
ΣM_i ________________________	g	ΣM_i^2 ________________________	g^2
$M_{average}$ ____________________	g		
$(\Sigma M_i)^2$ ____________________	g		
$(\Sigma M_i)^2 / n$ ________________	g		

(continued on next page)

Substitute your measured values in the two equations below.

Measured Volume (corrected) = ($M_{average}$ + $e_{average}$) × Z factor

________ mL = (________ + ________) × ________

% Accuracy = [(Measured Vol. – Expected Vol.) / Expected Vol.] × 100

________ % = [(________ – ________) / ________] × 100

Standard Deviation

$$SD = \sqrt{\frac{\sum M_i^2 - \frac{\left(\sum M_i\right)^2}{n}}{n-1}}$$

$$= \sqrt{\frac{(\qquad\qquad) - (\qquad\qquad)}{9}}$$

$$= \underline{\qquad\qquad\qquad}$$

% CV

$$\% \; CV = \frac{SD}{(\overline{M} + e_{average})} \times 100$$

= [________ / (________ + ________)] × 100

= ________

Pipette Precision Verification Worksheet
Example Calculations

Date ____________ **Technologist** ____________

Test Conditions

Pipette ID ____________

Expected Volume *1.0* mL*

Air Temperature *23.0* °C

Water Temperature *22.0* °C

Humidity *63.0* %

Barometric Pressure *29.4*

Z factor (from Appendix A) *1.0033*

Evaporation Measurement

Time		Evaporation	
t_1	*18* s	e_1	*0.0038* g
t_2	*16* s	e_2	*0.0026* g
t_3	*17* s	e_3	*0.0022* g
t_4	*16* s	e_4	*0.0034* g
$t_{average}$	*17* s	$e_{average}$	*0.0030* g

Weight Measurements

Mass		Mass^2	
M_1	*0.98501* g	M_1^2	*0.970245* g^2
M_2	*0.98895* g	M_2^2	*0.978022* g^2
M_3	*0.98766* g	M_3^2	*0.975472* g^2
M_4	*0.98660* g	M_4^2	*0.973380* g^2
M_5	*0.98522* g	M_5^2	*0.970658* g^2
M_6	*0.98523* g	M_6^2	*0.970678* g^2
M_7	*0.98700* g	M_7^2	*0.974169* g^2
M_8	*0.98627* g	M_8^2	*0.972729* g^2
M_9	*0.98420* g	M_9^2	*0.968650* g^2
M_{10}	*0.98691* g	M_{10}^2	*0.973991* g^2
ΣM_i	*9.86305* g	ΣM_i^2	*9.727994* g^2
$M_{average}$	*0.98631* g		
$(\Sigma M_i)^2$	*97.27976* g		
$(\Sigma M_i)^2 / n$	*9.727976* g		

* 1000µL = 1mL. To convert microliters to milliliters, divide microliters by 1000.

Substitute your measured values in the two equations below.

Measured Volume (corrected) = ($M_{average}$ + $e_{average}$) × Z factor

_*0.9926*_ mL* = (_*0.98631*_ + _*0.00300*_) × _*1.0033*_

% Accuracy = [(Measured Vol. – Expected Vol.) / Expected Vol.] × 100

_*-0.74*_ % = [(_*0.9926*_ – _*1.000*_) / _*1.000*_] × 100

Standard Deviation

$$SD = \sqrt{\frac{\sum M_i^2 - \frac{\left(\sum M_i\right)^2}{n}}{n-1}}$$

$$= \sqrt{\frac{9.727994 - 9.727976}{9}}$$

= _*0.0014142*_

% CV

$$\% \, CV = \frac{SD}{(\overline{M} + e_{average})} \times 100$$

= [_*0.0014*_ / (_*0.986305*_ + _*0.0030*_)] × 100

= _*0.14*_ %

* 1000µL = 1mL. To convert microliters to milliliters, divide microliters by 1000.