Ovation® BioNatural Pipettes

Performance Verification Protocol

Introduction

This document describes the recommended protocol for verifying performance of all models of the Ovation® BioNatural Pipette. The method utilizes gravimetric measurements to determine accuracy and precision, and should be followed exactly using Ovation pipette tips and the specified ancillary equipment in order to achieve published performance claims.

If access to the specified equipment is not available, VistaLab Technologies, Inc. maintains a qualified service and repair center which can perform repair service and calibration verification. Contact VistaLab Technical Services for more information.

Required Materials for Performance Testing

Balance	Balance should be capable of weighing to a minimum of 5 (0.01mg or 0.00001g) or 6 decimal places (0.001mg or 0.000001g) depending on the volume being tested. The sensitivity of the balance should be as follows: Volume — Sensitivity 10µL or less — 0.001mg >11µL — 0.01mg • Balance should be regularly serviced and certified by a qualified technician, using weights traceable to the National Institute of Standards and Technology (NIST). Between service calls, balance should be qualified using NIST traceable weights; and they should be confirmed for stability, integration time, and levels. • Balance should be stationed on marble tables or balance tables mounted on elastomeric vibration isolator pads to minimize vibration. • Balance environment should be humidified in order to prevent evaporation of the dispensed test volume. • Balances should be turned on at least one hour prior to use.
Weighing Vessels	Weighing vessels should be narrow mouthed with a diameter to height ratio of approximately 1:3. Volume capacity should be a minimum of 10 times the test volume. In an especially arid climate, vessels with covers to minimize evaporation may be appropriate.
Thermometer	Thermometer should be calibrated and readable to 0.1°C to measure the temperature of the water.
Pipette Tips	Tips used should be Ovation® pipette tips manufactured by VistaLab Technologies, Inc.
Hygrometer	Calibrated hygrometer to measure the environment humidity unless humidity is known to be within range of 45-75%.
Water	Non-aerated deionized or distilled water that has been allowed to equilibrate to room temperature in an appropriate container for at least two (2) hours prior to testing.
Stopwatch	Stopwatch is used to determine cycle time during the evaporation testing.

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When to Verify

All Ovation pipettes manufactured and serviced by VistaLab Technologies, Inc. are shipped with a calibration certificate that is traceable to NIST.

It is recommended that Ovation pipettes be verified for accuracy and precision whenever any of the following conditions occur:

1. Routinely, every six (6) months
2. If quality control samples suggest
3. If any maintenance, other than cleaning of the outer surfaces or changing the nozzle with filter, has been performed

Some laboratory-specific procedures call for an internally performed verification prior to placing any new pipetting device into routine use.

Environment

A controlled environment is necessary to ensure test reliability. Fluctuations in room temperature and humidity will adversely affect data. Maintain the following laboratory conditions for at least two (2) hours prior to, and throughout, the verification procedure. For traceability, use a temperature and humidity chart recorder for measuring conformity to specifications.

Ensure that balances, pipettes, and tips are properly equilibrated to ambient conditions. Allow them to equilibrate at least two (2) hours prior to verification.

Temperature	Air: 21.5 ± 1°C measured to .1° C Water: 21.5 ± 1°C measured to .1°C
Relative Humidity	45-75%
Barometric Pressure	measured to ±20mmHg, 25mbar, 0.15kpa, or 0.7inHg
Conditioning	Keep the room air circulating fan running continuously to prevent temperature surges. Drafts should be minimized and balance should not be located in a drafty location.
Lighting	Use diffused light of sufficient intensity. Avoid direct sunlight, which may cause a local rise in temperature thereby affecting results.

Pipette Operation

The Ovation BioNatural Pipette is an air displacement, single or two stroke pipette intended to aspirate and dispense precise fluid volumes. Ovation pipettes with the adjustable volume feature aspirate and dispense with two stroke (overblow) operation. Ovation pipettes with fixed volume settings are available with either single or two stroke operation (check external labeling).

When pipetting, it should be held so that the nozzle and tip are nearly vertical (0-30°). Pipetting consistency and proper holding 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.

Set the pipette to the desired volume. For optimum performance over the entire pipetting range, set the pipette volume to the nominal (highest) volume. For optimum performance at a specific volume for a specific fluid, set the pipette volume to the desired volume for the specific fluid.

Humidity Equilibrium Prior to Testing

Before any testing is performed, the dead air volume within Ovation's interior needs to reach humidity equilibrium using the following procedure:

1. Set the Ovation to the test volume setting.
2. Place a new dry tip on the nozzle.
3. Place the tip in the water and aspirate and dispense five (5) times. Discard the sample or dispense back into the sample reservoir.
4. Discard the tip.
5. Install a new tip, pre-rinse the tip and immediately begin to collect data.

Immersion Depth

When pipetting, tips should be immersed according to the following depth recommendations. Immersing a tip too deeply in a sample forces additional liquid (that is not part of the measured volume) into the pipette tip. This liquid can be incorrectly dispensed along with the measured volume. Also, there is the increased possibility of water carryover on the outside of the tip. Immersing the tip too shallow may cause air bubbles to be aspirated into the pipette tip.

Tip Immersion Depth

0.2µL	to	10µL	1mm
11µL	to	100µL	2-3mm
101µL	to	1000µL	2-4mm

Pre-Rinsing the Tip

A new, dry tip should be used for each dif ferent volume setting to be tested.

1. Set the Ovation pipette to the desired volume setting.
2. Place the tip in the water to the required immersion depth and aspirate and dispense one (1) time. Discard the sample or dispense back into the sample reservoir.
3. Aspirate and dispense according to the testing protocol and collect data.
4. If air bubbles are seen in the tip during pre-rinsing or during data collection, discard the tip and install a new, dry tip. Pre-rinse this new tip before using.

To Aspirate and Dispense

1. On two stroke models, press the plunger down to the first stop; on single stroke models, fully depress the plunger. Immerse pipette tip in the sample.
2. Smoothly and slowly, release the plunger allowing sample to enter the pipette tip. W ait one second before withdrawing the tip from the sample.
3. Place the pipette tip against the side of the receiving vessel close to the bottom of the vessel, or if it contains liquid, just above the surface of the liquid.
4. On two stroke models, smoothly press the plunger to the first stop, wait one second, then fully depress the plunger to the second stop to dispense all liquid from the tip. On single stroke models, fully depress the plunger.
5. With the plunger depressed, slowly withdraw the tip.
6. Release the plunger when the tip is away from the receiving vessel.

See "Pipetting Hints for Optimal Performance" for additional information.

Evaporation Rate

Evaporation is estimated by means of a series of simulated weighings to determine how much water weight is lost due to 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 (water) weight is lost during the elapsed time.

A low humidity environment and/or if the pipetting cycle is unusually long, will have an adverse ef fect on the performance data. Due to the increased loss of water weight, pipettes will appear to be reading lower than the intended specifications. For these reasons, it is important to maintain a consistent time and humidified environment when performing pipette verification. This is especially critical when testing any pipette at low volume settings.

Measurement Timing Procedure

1. Fill an appropriate weighing vessel at 1/4 to 1/3 full with room temperature equilibrated water, and place it on the balance.
2. Start the stopwatch and perform a normal weighing cycle. Stop the stopwatch when the balance has settled after the sample is added.
3. Repeat the timing check for a total of four measurements, and calculate the average time. Record this average Evaporation Time on worksheet.

Evaporation Measurement Procedure

1. Perform a simulated weighing, however, do not dispense the water from the tip into the weighing vessel on the balance. Instead, dispense the water back into the water reservoir or discard it to waste.
2. Record the weight loss that has occurred at the end of the average Evaporation Time determined previously.
3. Repeat for a total of four measurements, 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 (mg) when calculating volume.

Note: Recalculate the evaporation rate every four (4) hours or whenever ambient conditions change.

Verification Procedure

Based on industry guidelines and recommendations, any pipette that is tested by other than the original instrument manufacturer should be tested using at least ten (10) data points for performance verification. Follow the recommendations and directions given above, and:

1. Determine the average estimated evaporation weight
2. Perform ten (10) water weighings
3. Using the worksheet contained later in this document, in conjunction with laboratory software or a calculator, determine the mean volume, accuracy (±%) and precision (CV%).
4. Recalibrate the Ovation pipette using the Ovation's calibration software, if necessary. Refer to the Operator's Guide for additional instructions.

Test Results

Record the results of each test including the test conditions as discussed in "Environment" earlier. The data should include:

A. Test Conditions

1. Ambient Air Temperature
2. Water Temperature
3. Humidity
4. Barometric Pressure
5. Z Factor (The Z factor is required in the volumetric calculations to compensate for the density of the water at the test conditions. See Appendix A)

B. Measured Mean Evaporation *e*

C. 4 or 10 Individual Mass Readings

D. Following Values Should Be Calculated:

1. **Mean Measured Mass,** calculated as:

$$\frac{\text{(sum of individual weight measurements)}}{\text{(number of readings)}}$$

2. **Measured Volume (corrected),** calculated as:

(Mean Mass + Mean Evaporation) x (Z Factor) (see Appendix A)

3. **% Accuracy (**for precision and accuracy test), calculated as:

$$\frac{\text{(Measured Volume – Expected Volume)}}{\text{Expected Volume}} \times 100$$

4. **Standard Deviation** (for precision test), calculated as:

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

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:

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

5. **% CV** (for precision test), calculated as:

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

Where:

$e_{average}$ = evaporation average in grams

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The procedures contained in this protocol are based on pipette verification recommendations from the following sources: DIS; NCCLS; GLP; V istaLab Technologies, Inc.; Ovation BioNatural Pipette Operator's Guide

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 ∫C and 680 mm Hg = 1.0024 Z factor).

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 (N CCLS), May 1984

Pipette Accuracy and Precision Verification Worksheet

Ovation BioNatural Pipette

Test Conditions

Pipette ID _____

Expected Volume _____µL

Air Temperature _____°C

Water Temperature _____°C

Humidity _____%

Barometric Pressure _____

Balance Serial No. _____

Model _____

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		

Determine Percent % Accuracy

Substitute your measured values in the two equations below.

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

_____ µL = (_____ + _____) x _____

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

_____ % = [(_____ – _____) / _____] x 100

Determine Standard Deviation

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

$$SD = \sqrt{\frac{(\quad) - (\quad)}{9}}$$

= _____

Determine % CV

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

= [_____ / _____ + _____] x 100

= _____

Performed by: _____

Date: _____

Pipette Accuracy and Precision Verification Worksheet

Ovation BioNatural Pipette

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

Balance Serial No. _____

Model _____

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		

Determine Percent % Accuracy

Substitute your measured values in the two equations below.

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

0.9926 µL = (0.98631 + 0.0030) x 1.0033

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

– 0.74 % = [(0.9926 – 1.000) / 1.000] x 100

Determine Standard Deviation

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

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

= 0.0014142

Determine % CV

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

= [0.0014 / 0.986305 + 0.0030] x 100

= 0.14

Performed by: _____

Date: _____

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