Procedure for Evaluating Accuracy and Precision of Rainin Pipettes

Factory-Approved Method for Using Gravimetric Analysis



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For Technical Information, call 800-543-4030 FAX: 781-938-1152

For Service Information, call 800-662-7027 FAX: 781-935-7631

For detailed information on troubleshooting and repairing Pipetman, request Rainin publication **AB-14**, *Pipetman Care & Maintenance*, or download from website.

Scope

This document describes the factory-approved method for determining pipette performance using gravimetric analysis. Adherence to this method by Rainin's Pipette Service Department assures conformity to original manufacturer's conditions and specifications.

The document covers two methods:

- 2 Volumes x 4 Weighing Factory Method confirms pipette accuracy and precision for new pipettes It is also used for pipettes serviced following recalibration and repair through Rainin's Express Repair and Performance Verification programs.
- 3 Volumes x 10 Weighing Method confirms pipette accuracy and precision for pipettes serviced through Rainin's Performance Verification program.

These procedures are applicable to all Rainin and Pipetman pipettes and have been adopted for use with other manufacturer's pipettes.

DEFINITIONS

accuracy: The closeness of a measured volume to the true volume as specified by the volume setting of the pipette. Also known as "mean error".

calibration: The fine adjustment of the piston stroke length for mechanical pipettes.

evaporation rate: An estimate of water loss due to evaporation during the weighing procedure. Evaporation rates are calculated when evaluating pipettes using 6- and 7-place balances. See *Microvolume Procedures* (page 7) for more information.

gravimetric analysis: The general procedure based upon the determination of the weight of water samples as delivered by the pipette. Values are corrected for evaporation, then true mass and volume are calculated simultaneously, based upon the knowledge of the density of water at specific temperatures with corrections for air buoyancy (Z-factor).

precision: The closeness of agreement among the individual weighings. Also known as standard deviation, reproducibility and repeatability.

prerinse: A double rinse of a pipette tip with testing medium. Repeat prerinse whenever a new tip is applied or weighing cycle time has been interrupted.

Z-factor: Conversion factor (μ L/mg) incorporating the density of water when buoyed in air as a function of temperature and pressure. For Rainin's laboratory conditions, the Z-factor is 1.0031 at 1 A.T.M. (See table on page 4).

CALCULATIONS

MEAN WEIGHT: Result expressed in mg.	$\overline{w} = \sum_{i=1}^{n} w_{i}$ $\overline{w} = \text{mean weight}$ $n = \text{number of measurements}$ $w_{i} = \text{individual weighings}$
MEAN VOLUME : The mean weight result with corrections for evaporation and Z-factor. Expressed in μL.	$\overline{v} = (\overline{w} + \overline{e}) \times Z$ $\overline{w} = \text{mean weight (mg)}$ $\overline{v} = \text{mean volume}$ $\overline{e} = \text{evaporation rate (mg)}$ Z = Z-factor
MEAN ERROR: The difference between the mean volume of actual measurements and the true value as specified by the volume setting of the pipette. Expressed in µL.	$E = \overline{v} - v_{o}$ $E = \text{mean error}$ $\overline{v} = \text{mean volume}$ $v_{o} = \text{volume setting}$
As a percentage	$E \% = \frac{v - v_0}{v_0} \times 100$
STANDARD DEVIATION: Quantifies the magnitude of scatter due to random error.	$s = \sqrt{\sum_{i=1}^{n} (\overline{w} - w_i)^2}$ $n - 1$ $s = standard deviation$ $n = number of weighings$ $\overline{w} = mean weighing$ $w_i = individual weighings$

As a percentage, also known as coefficient of variation (CV)

$$S\% = \frac{S}{\bar{v}} \times 100$$

Laboratory

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

- Temperature 21.5 ± 1.0°C
- Relative Humidity 45–75%
- Conditioning
 Keep the room circulating fan permanently on to prevent temperature surges. Drafts should be minimized.
 Illumination
 Use diffused light of sufficient intensity.
- Avoid direct sunlight.
 Chronicle
 Use a temperature and humidity chart recorder for measuring conformity to specifications. Retain records for traceability.

Testing Medium

Non-aerated deionized water is used as the standard in gravimetric analysis.

Value for Z (µL/MG), as a Function of Temperature and Pressure, for Distilled Water at 1 atm.

TEMP °C	ACTUAL Z-FACTOR	TEMF °C	P ACTUAL Z-FACTOR	2	TEMP °C	ACTUAL Z-FACTOR
15.0 15.5 16.0 16.5 17.0 17.5 18.0	1.0020 1.0020 1.0021 1.0022 1.0023 1.0024 1.0025	20.0 20.5 21.0 21.5 22.0 22.5 23.0 23.5	1.0029 1.0030 1.0031 1.0032 1.0033 1.0034 1.0035		25.0 25.5 26.0 26.5 27.0 27.5 28.0 28.5	1.0040 1.0041 1.0043 1.0044 1.0045 1.0047 1.0048
18.5 19.0 19.5	1.0026 1.0027 1.0028	23.5 24.0 24.5	1.0036 1.0038 1.0039		28.5 29.0 29.5 30.0	1.0050 1.0051 1.0052 1.0054

Fluctuation in room temperature and humidity will adversely affect balance stability and evaporation rates. A stringently-controlled environment assures reliable data.

Balances

Appropriate 4-, 5-, 6-, and 7-place balances are selected to measure pipette performance. The sensitivity of the balance chosen must be equal to or less than one-tenth of the smallest deviation to be assessed.

		APPLICABLE MODELS BY
<u>SENSITIVITY(g)</u>	DISPLAY	<u>NOMINAL VOLUME (µL)</u>
10 ⁻⁷	0.0000 mg	2, 10
10 ⁻⁶	0.000 mg	20, 25
10 ⁻⁵	0.00 mg	100, 200, 250
10-4	0.0 mg	1000, 2000, 2500, 5000, 10 mL

Work Stations

Balances are stationed on marble tables to minimize vibration. A black mat is positioned in front of the weighing chamber to expose droplets and create a cleaner environment. Each balance is directly coupled with a computer and Rainin software for data acquisition, statistical computation, and report production. Balances are regularly serviced and certified by Mettler technicians.

Balance Certification

Balances are regularly serviced and certified by Mettler technicians using weights traceable to the National Institute of Standards and Technology (NIST). Rainin senior technicians routinely qualify balances between service calls using NIST-traceable weights and confirm balance parameters such as stability, integration time and levels.

Tips

Rainin pipettes are designed and calibrated using Rainin FinePoint[™] and LTS[™] tips. Failure to use the original manufacturer's recommended tips may result in poor pipette performance.

Using a 4-place balance to calibrate a Pipetman P-20 can result in error three times greater than the pipette specification. Why? The 4-place balance inaccuracy is significantly larger than the inaccuracy of a P-20. Always use the right balance for the job.

Weighing Vessels

Cylindrical weighing vessels are constructed of non-porous plastic with flat bottoms. Some vessels have covers to minimize evaporation. Rubber O-rings prevent the transfer of heat from hands. The micro-volume vessel should be handled using forceps only. See page 7 for more information. A Weighing Kit consisting of all three vessel types and forceps is available and recommended.(Catalog No. **WK-1**)



Weighing Kit Catalog No. WK-1

Controlling evaporation is essential when evaluating micro-volume pipettes. Rainin uses a custom-designed micro-volume vessel to control this variable.

Evaluating micro-volume pipettes (i.e. less than 50 μ L) requires special procedures and equipment. This procedure must be followed whenever 6- and 7-place balances are used for determining pipette performance. The goal is to minimize, control and quantify evaporation loss during testing.

Two Vessel Method

Two micro-volume vessels with covers are used for each 6- and 7-place balance. Partially fill one vessel with approximately 100 μ L of water. Set the other vessel aside. Water should cover the entire bottom surface of the first vessel. This vessel may now be used for weighings. During testing, droplets accumulating on the inner wall should be mixed into the 100 μ L of water frequently.

When approximately 1/3 full, discard the entire contents of the first vessel, dry it with a lint-free material, and set it aside. Retrieve the unused vessel and partially fill with water as described above. Alternate vessels throughout the entire evaluation process or whenever sample is splashed on the cover.

Estimating the Evaporation Rate

Evaporation is estimated by running a series of four simulated weighings, only duplicating the weighing cycle without the addition of sample liquid to the vessel. Measure the total weight difference due to evaporation and divide by 4 to obtain an average. The rate is expressed in mg.

SIMULATED WEIGHING PROCEDURE

- 1. Fill the micro-volume vessel one-third full with water.
- 2. Cover the vessel and place in balance using forceps.
- 3. Using a pipette, aspirate a sample at full volume setting from the reservoir.
- 4. Tare the balance and remove the vessel.
- 5. Remove cover with forceps.
- 6. Dispense sample into the reservoir, not the micro-volume vessel.
- 7. Cover the micro-volume vessel and return it to the balance.
- 8. Record the result e1 (negative value).
- 9. Repeat steps 3 through 8 three times to obtain e2, e3 and e4.
- 10. Calculate the rate: $\mathbf{\bar{e}} = -(\mathbf{e}_1 + \mathbf{e}_2 + \mathbf{e}_3 + \mathbf{e}_4)/4$
- 11. Round to the nearest 0.005 mg, and convert to a positive value.
- 12. The evaporation rate **ē** should be added when calculating the mean volume of each real sample weighing.

Evaporation rates usually range between 0.010 – 0.025 mg per weighing cycle. Recalculate the evaporation rate every 4 hours or whenever ambient conditions change.

Qualifying a Technician

To qualify for assessing pipette performance a technician must first successfully complete three different proficiency trials in sequence for each pipette model.

■ **Precision Trial:** Using an accurate pipette, take thirty weighings at each volume setting. Volume settings are generally 10, 50 and 100% of nominal. Calculate the standard deviation. If results are 1/3 less than precision specifications (min. 3 consecutive pipettes), continue to the next trial.

■ Accuracy Trial: Take four weighings at each volume setting. Volume settings are generally 10, 50 and 100% of nominal. Calculate the mean volume. If comparative mean differences are consistently less than 1/3 of the accuracy specification (min. 3 consecutive pipettes), continue to the next trial.

■ Calibration Trial: Using a number of purposely miscalibrated mechanical pipettes, the trainee calibrates each pipette according to Rainin's calibration procedure. A senior technician then re-verifies each unit for compliance to published specification. If a minimum of 14 consecutive pipettes pass, the trainee is qualified to evaluate and calibrate this model. Repeat procedure for other models/volume ranges.

Pipette Operation

Consistency in all aspects of a pipetting procedure will significantly contribute to reproducibility.

1. When picking up a sample, immerse the end of the disposable tip beneath the liquid surface within the following ranges:

IMMERSION	APPLICABLE
<u>DEPTH (mm)</u>	MODELS
1-2	up to 10 μL
2-3	10 to 100 μL
2-4	100 to 1000 μL
2-6	1000 to 2000 µL
6-10	2000 µL or more

- 2. Prerinse tips whenever critical reproducibility is required.
- 3. Maintain consistency in the following areas:
 - pipetting rhythm from sample to sample.
 - speed and smoothness when you press and release the push button.
 - push button pressure at the first stop.
 - angle and immersion depth.
- 4. Always operate a pipette in a vertical position.
- 5. Dispense sample by touching the tip end against the side wall of the receiving vessel to ensure complete sample flow.

With Pipetman pipettes, always wind the micrometer 1/3 revolution above the desired volume setting and then return clockwise. This will minimize errors in volume delivery due to mechanical backlash.

This method confirms pipette accuracy and precision for new pipettes and for pipettes serviced through Rainin's Express Repair and Performance Verification programs. Many customers consider this method satisfactory for periodically checking pipette performance. Conditions, procedures and qualifications previously described in this document should be implemented to assure validity of test results.

Description

After prerinsing the tip, record four individual weighings per volume setting. Use a new tip for each volume setting. Two volume settings should be tested for each model, beginning with the minimum setting first, as specified in the table below. (Testing at any one volume is not sufficient to assess proper calibration of a variable volume pipette.) Holding or hand warming the pipette shaft should be avoided throughout the test procedure.

Volume Settings

1.	1. For new pipettes and pipettes serviced through Rainin Express Repair.							
	PIPETTE <u>TYPE</u>	PIPETTE MODEL BY NOMINAL VOLUME	VOLUN % OF NO <u>MIN</u>	ME SET OMINAL <u>MAX</u>				
	Rainin	All models	10%	100%				
2.	Pipetman For pipetter Calibration	10, 20, 10ML, and 200-M8 100, 1000, 5000 2, 200 s serviced through the Verifica Plan of Rainin's Performance	10% 20% 25% ation and Verificati	100% 100% 100%				
	program.							
	PIPETTE <u>TYPE</u> Rainin	PIPETTE MODEL BY <u>NOMINAL VOLUME</u> All models	VOLUN % OF No <u>MIN</u> 10%	ME SET OMINAL <u>MAX</u> 100%				
	Pipetman	All models except 200 200	10% 25%	100% 100%				

Results

Calculate the mean volume and standard deviation for each volume setting using the formulas on page 3. Compare results to specifications on pages 13-14 and determine conformity.

Individual weighings are calculated by subtracting the balance tare reading from the sample reading. Consistent rhythm during weighing operations should be maintained.

This method confirms pipette accuracy and precision for pipettes serviced through Rainin Performance Verification and Extended Calibration program. Customers requiring higher confidence levels of measured results prefer this method. Conditions, procedures, and qualifications previously described in this document should be implemented to assure validity of test results.

Description

After prerinsing the tip, record ten individual weighings per volume setting. Change tips between each volume setting. Three volume settings are selected per pipette model based on the pipette's working range as specified in the table below. Holding or hand warming of the pipette shaft should be avoided throughout the procedure.

Volume Settings

Verification	Measurement Plan of Rain	in's Per	forman	ice
		VO	LUME	SET
PIPETTE	PIPETTE MODEL BY	% OI	F NOM	INAL
<u>TYPE</u>	NOMINAL VOLUME	MIN	MID	<u>MAX</u>
Rainin	All models	10%	50%	100%
Pipetman	All models except 200	10%	50%	100%
•	200	25%	50%	100%

Results

Calculate the mean volume and standard deviation for each volume setting using the formulas on page 3. Compare results to specifications on pages 13-14 to determine conformity.

While the 3 volumes x 10 weighing method provides a higher confidence level of measured results, the cost in time and money is often unwarranted when a pipette is subjected to preventive maintenance, a standard feature of Rainin's Performance Verification program. To learn more, request Technical Report 9804, *Comparison of Ten vs Four Weighing Method*, or download from the website.

2 Volumes x 4 Weighing Factory Method

New Rainin Pipette

Identification	n	G	ravimetric Det	8
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Carlos Tap races			18.08	198.00
			18.91	100.54
		5-0-11-11-1	20.04	198.58
Operating Cond	itions	Mean ingi-	19.90	186.83
Technislan.	TK .	Maam (uit)	29.64	198.15
Optigration Hat.	ACHI	Procession (and)	0.044	0.112
Philame Samplivity (g)	0.00804		the state	
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Correction for Evaporation.	0.000	Low Volume 3milling Lot	26	Pes
Temperatum 101	21.8 ± 14	Fight Walkering Solding Lott	208	Pase
Farverwood Optia has Deer	onwerlad from r	ng to pill for disalitant water. This y	hibergé ve ai couvo	niy wik speciesters.

Performance Verification Pipette

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TEMPERATURE HUMBLIN			PEPETTE MODEL SERVAL # HITERMAL ID	PRETLITE 200 CREVIE
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3 Volumes x 10 Weighing Method

Performance Verification Pipette

INSTITUTION X NAME JA DEPT	YZ TECHNO VNE SIVITH	alogy		PIPETTE M SERIAL # INTERNAL	IDDEL	PIPET LITE 200 D90613 0864
TEMPERATURE HUMIDITY EVAPORATION RAT CERTIFIED TIPS	TE (mg)	CONE 21.5 °C (st.0) 51% 0 LTS-250	DITIONS AN	D EQUIPMENT	MLANCE MODEL SERIAL # SENSITIVITY (G) TRACEABILITY	AT261 1117023801 00001 NIST
INSPECTION TEST PREVENTIVE MAIN CORRECTIVE ACT	ITENANCE	Pass Replaced seal, cleaner Calibration confirmed	d piston			
			AS F	OUND		
MEAN (µl) PRECISION (est) STATUS		20 µl 20.46 03 Pass	10 00 Pa	00 pil 0.22 1 199	200 200.2 .02 Pass	2 ¹¹
-			AS RE	TURNED		
WEIGHENGS (mg)	20.02 20.08 20.1 20.01 20.03	20 µl 20.09 20.05 20.06 20.12 20.00	100.08 100.13 100.15 100.24 100.24	100 µi 100.23 100.33 100.3 100.29 100.3	199.45 199.5 199.5 199.58 199.59 199.52	μl 199.58 199.75 199.68 199.73 199.75
RESULTS MEAN (µ) EPROR (%) PRECISION CV (%)	ACTUAI 20.13 0.65% .04 0.20%	SPECIFICATIONS 19.5 to 20.5 +- 2.5 0.2 1	ACTUAL 100.54 0.54% 08 0.08%	SPECIFICATIO 99.2 to 100.8 **- 8 25 25	NS ACTUAL 200.23 0.12% .11 0.05%	SPECIFICATION 196.4 to 201.6 +/8 0.3 .15
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SPECIFICATIONS

<u>PIPETMAI</u>	N				
MODEL	VOLUME	ACCU	RACY	PRECI	SION
VOLUME	SET µL	%	μL(±)	%	μL(≤)
P-2	0.2	12.0	0.024	6.0	0.012
	1	2.7	0.027	1.3	0.013
	2	1.5	0.030	0.7	0.014
P-10	1	2.5	0.025	1.2	0.012
	5	1.5	0.075	0.6	0.03
	10	1.0	0.1	0.4	0.04
P-20	2	7.5	0.15	2.0	0.04
	10	1.5	0.15	0.5	0.05
	20	1.0	0.2	0.3	0.06
P-100	10	3.5	0.35	1.0	0.1
	50	0.8	0.4	0.24	0.12
	100	0.8	0.8	0.15	0.15
P-200	50	1.0	0.5	0.4	0.2
	100	0.8	0.8	0.25	0.25
	200	0.8	1.6	0.15	0.3
P-200-M8	20	2.5	0.5	1.25	0.25
	100	1.0	1.0	0.5	0.5
	200	1.0	2.0	0.5	1.0
P-1000	100	3.0	3.0	0.6	0.6
	500	0.8	4.0	0.2	1.0
	1000	0.8	8.0	0.15	1.5
P-5000	500	2.4	12.0	0.6	3.0
	2500	0.6	15.0	0.2	5.0
	5000	0.6	30.0	0.16	8.0
P-10ML	1 mL	5.0	50.0	0.6	6.0
	5 mL	1.0	50.0	0.2	10.0
	10 mL	0.8	80.0	0.16	16.0

DISTRIMAN

MODEL	VOLUME	ACCU	RACY	PRECISION		
VOLUME	<u>SET μL</u>	%	μL(±)	%	μL(≤)	
125 µL TIP	2	5.0	0.10	4.0	0.08	
	5	2.5	0.125	1.5	0.075	
	10	2.0	0.20	1.0	0.10	
1250 µL TIP	20	4.0	0.80	1.0	0.2	
-	50	2.0	1.00	0.8	0.4	
	100	1.0	1.00	0.6	0.6	
12.5 mL TIP	200	3.0	6.0	0.5	1.0	
	500	1.5	7.5	0.3	1.5	
	1000	1.0	10.0	0.25	2.5	

MICROMAN

MODEL	VOLUME	ACCURACY		PRECIS	PRECISION	
VOLUME	<u>SET µL</u>	%	μL(±)	%	μ L(≤)	
M-10	1	6.0	0.06	2.0	0.02	
	5	2.5	0.10	0.6	0.03	
	10	1.5	0.15	0.5	0.05	
M-25	3	5.0	0.15	2.0	0.06	
	10	1.7	0.17	0.8	0.08	
	25	1.0	0.25	0.4	0.10	
M-50	20	1.7	0.35	0.6	0.12	
	50	1.0	0.50	0.3	0.15	
M-100	10	5.0	0.50	2.0	0.20	
	50	1.5	0.75	0.6	0.30	
	100	1.0	1.00	0.4	0.40	
M-250	50	3.0	1.50	0.4	0.20	
	100	1.7	1.70	0.3	0.30	
	250	1.0	2.50	0.2	0.50	
M-1000	200	1.5	3.00	0.8	1.60	
	500	1.0	5.00	0.5	2.50	
	1000	0.8	8.00	0.4	4.00	

RAININ PIPET-LITE, PIPET-PLUS, AND EDP3

MODEL	VOLUME	ACCURACY		PRECIS	PRECISION	
VOLUME	<u>SET μL</u>	%	μL(±)	%	μ L(≤)	
2	0.2	12.0	0.024	6.0	0.012	
	1	2.7	0.027	1.3	0.013	
	2	1.5	0.030	0.7	0.014	
10	1	2.5	0.025	1.2	0.012	
	5	1.5	0.075	0.6	0.03	
	10	1.0	0.1	0.4	0.04	
20	2	7.5	0.15	2.0	0.04	
	10	1.5	0.15	0.5	0.05	
	20	1.0	0.2	0.3	0.06	
100	10	3.5	0.35	1.0	0.1	
	50	0.8	0.4	0.24	0.12	
	100	0.8	0.8	0.15	0.15	
200	20	2.5	0.5	1.0	0.2	
	100	0.8	0.8	0.25	0.25	
	200	0.8	1.6	0.15	0.3	
1000	100	3.0	3	0.6	0.6	
	500	0.8	4	0.2	1.0	
	1000	0.8	8	0.15	1.5	
2000	200	3.0	6	0.60	1.2	
	1000	0.8	8	0.20	2.0	
	2000	0.8	16	0.12	2.4	
5000	500	2.4	12	0.60	3	
	2500	0.6	15	0.20	5	
	5000	0.6	30	0.16	8	
10ML	1 mL	5.0	50	0.60	6	
	5 mL	1.0	50	0.20	10	
	10 mL	0.8	80	0.16	16	

RAININ EDP, EDP2, AND EDP-PLUS

MODEL	VOLUME	ACCURACY		PRECI	PRECISION	
VOLUME	<u>SET µL</u>	%	μL(±)	%	μ L(≤)	
10 µL	1	2.5	0.025	1.2	0.012	
(E2 & EP only	y) 5	1.5	0.075	0.6	0.03	
	10	1.0	0.1	0.4	0.04	
10 µL	1	10.0	0.1	4.0	0.04	
(black EDP only) 5		2.0	0.1	2.0	0.04	
	10	1.0	0.1	0.5	0.05	
25 µL	2.5	6.0	0.15	2.0	0.05	
	12.5	1.2	0.15	0.4	0.05	
	25	1.0	0.25	0.3	0.075	
100 µL	10	3.0	0.3	1.0	0.1	
	50	0.8	0.4	0.2	0.1	
	100	0.8	0.8	0.2	0.2	
250 µL	25	2.0	0.5	0.6	0.15	
	125	0.8	1.0	0.15	0.19	
	250	0.8	2.0	0.15	0.38	
1000 µL	100	3.0	3.0	0.6	0.6	
	500	0.8	4.0	0.2	1.0	
	1000	0.8	8.0	0.15	1.5	
2500 μL	0.25 mL	3.2	8.0	0.8	2.0	
	1.25 mL	0.8	10.0	0.16	2.0	
	2.5 mL	0.8	20.0	0.12	3.0	
10ML	1 mL	5.0	50.0	0.6	6.0	
	5 mL	1.0	50.0	0.2	10.0	
	10 mL	0.8	80.0	0.16	16.0	



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