



US Environmental Protection Agency Office of Pesticide Programs

**Office of Pesticide Programs
Microbiology Laboratory
Environmental Science Center, Ft. Meade, MD**

Standard Operating Procedure for Verification of Digital Pipettes

SOP Number: QC-19-09

Date Revised: 03-05-18

SOP Number	QC-19-09
Title	Verification of Digital Pipettes
Scope	Describes process for verification of digital pipettes.
Application	Pipettes are calibrated annually by an ISO 17025 accredited vendor and are verified in-house using the gravimetric procedure as necessary.

	Approval	Date
SOP Developer:	_____	_____
	Print Name: _____	
SOP Reviewer	_____	_____
	Print Name: _____	
Quality Assurance Unit	_____	_____
	Print Name: _____	
Branch Chief	_____	_____
	Print Name: _____	

Date SOP issued:	
Controlled copy number:	
Date SOP withdrawn:	

TABLE OF CONTENTS

<u>Contents</u>	<u>Page Number</u>
1. DEFINITIONS	3
2. HEALTH AND SAFETY	3
3. PERSONNEL QUALIFICATIONS AND TRAINING	3
4. INSTRUMENT CALIBRATION	3
5. SAMPLE HANDLING AND STORAGE	3
6. QUALITY CONTROL	3
7. INTERFERENCES	3
8. NON-CONFORMING DATA	3
9. DATA MANAGEMENT	3
10. CAUTIONS	3
11. SPECIAL APPARATUS AND MATERIALS	3
12. PROCEDURE AND ANALYSIS	4
13. DATA ANALYSIS/CALCULATIONS	5
14. FORMS AND DATA SHEETS	5
15. REFERENCES	5

1. Definitions	Abbreviations/definitions are provided in the text. <ol style="list-style-type: none"> 1. Verification failure = Verification of pipette exceeds $\pm 5\%$ of the target volume. 2. Ideal volume = Target volume; actual volume being measured, corrected for temperature using the density of water at 21.0°C (0.997995 g/mL, see section 15.1).
2. Health and Safety	Follow procedures specified in SOP MB-01, Laboratory Biosafety. The Study Director and/or lead analyst should consult the Safety Data Sheet for specific hazards associated with products.
3. Personnel Qualifications and Training	Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
4. Instrument Calibration	Refer to SOP EQ-03 (weigh balances) for details on method and frequency of calibration.
5. Sample Handling and Storage	None.
6. Quality Control	For quality control purposes, the required information is documented on the appropriate form(s) (see section 14).
7. Interferences	None.
8. Non-conforming Data	<ol style="list-style-type: none"> 1. Manage non-conforming data consistent with SOP ADM-07, Non-Conformance Reports. 2. Do not use pipettes if the inaccuracy exceeds $\pm 5\%$ of the target volume. For corrective actions, see section 12.3.
9. Data Management	<ol style="list-style-type: none"> 1. Archive data consistent with SOP ADM-03, Records and Archives. 2. Maintain an inventory of pipettes electronically using a Microsoft Excel spreadsheet (refer to section 14). After each addition to or deletion from the inventory, file a hard copy of the pipette inventory in the Pipette Verification and Calibration Record Book.
10. Cautions	<ol style="list-style-type: none"> 1. If a pipette is dropped or damaged, successfully verify it in-house using the gravimetric procedure prior to use. 2. If a pipette fails an in-house verification assessment, do not use it until it has been repaired (if necessary) and recalibrated by an ISO 17025 accredited vendor.
11. Special Apparatus and	<ol style="list-style-type: none"> 1. <i>Calibrated balances.</i> Capable of measuring 0.01 g for verifying pipettes with volumes greater than or equal to 1 mL and 0.001 g for verifying

<p>Materials</p>	<p>pipettes with volumes less than 1 mL.</p> <p>2. <i>Pipettes.</i></p> <ul style="list-style-type: none"> a. Rainin Adjustable Volume Pipettes b. Gilson Microman Positive Displacement Pipettes c. Gilson Distriman Continuously Adjustable Volume Repetitive Pipette d. Eppendorf Repeater Pipette
<p>12. Procedure and Analysis</p>	
<p>12.1 Pipette Verification Requirements</p>	<ul style="list-style-type: none"> a. Annually verify and service pipettes using an ISO 17025 accredited vendor. b. If a pipette is dropped or broken, verify the pipette using the gravimetric verification procedure (see section 10). c. Record the annual verification results and if necessary, gravimetric analysis results on the Pipette Verification Record Sheet (refer to section 14) and file in the Pipette Verification and Calibration Log Book. The Pipette Verification Record Sheet is based on the inventory of pipettes and may change over time.
<p>12.2 Gravimetric Verification Procedure</p>	<ul style="list-style-type: none"> a. Record all pertinent information for the gravimetric verification procedure on the Pipette Verification – Gravimetric Analysis Form (refer to section 14). b. In advance of testing, fill a container with de-ionized water and allow it to equilibrate to room temperature in the same laboratory with the balance. List the balance that will be used on the appropriate form. c. Place a small container on the balance and record its mass on the appropriate form. d. Using the pipette to be verified, aspirate an aliquot of DI water from the sample aliquot container and dispense into the small container. e. Record the mass on the appropriate form. f. Follow the procedure in 12.2d-e for each subsequent sample addition; measure at least 5 samples. Do not tare between samples. After each new sample addition, record the mass on the appropriate form. g. Input the measurements and other appropriate information into the Pipette Verification – Gravimetric Analysis Spreadsheet.

	<p>h. Verify that the percent inaccuracy is within $\pm 5\%$. If the percent inaccuracy is outside of this range, remove the pipette from use until it is repaired and calibrated by a vendor.</p> <p>i. $Percent\ Inaccuracy = \frac{(\bar{x} - ideal\ volume) \times 100}{ideal\ volume}$</p>								
12.3 Pipette Verification Record Sheet	<p>a. From the data on the calibration certificate for each pipette, record the following on the Pipette Verification Record Sheet:</p> <p>i. <i>Verification Status</i>: record as “pass” or “fail.” Record status as “pass” if the percent inaccuracy from gravimetric analysis is within $\pm 5\%$ or if vendor calibration certificate indicates acceptable results, otherwise record as “fail.”</p> <p>ii. <i>Corrective Action</i>: record as “yes” or “no.” If the verification status is “pass,” record as “no.” Record as “yes” if the verification status is “fail,” describe the action taken at the bottom of the page, and notify the Quality Assurance Officer (QAO). The QAO must determine if there was any impact on work conducted using the failed pipet.</p> <p>b. The Pipette Verification Record Sheet may be completed electronically or by hand.</p>								
13. Data Analysis/ Calculations	1. None								
14. Forms and Data Sheets	<p>1. Sample Pipette Verification Record Sheet</p> <p>2. Sample Pipette Inventory</p> <p>3. Test Sheets. Test sheets are stored separately from the SOP under the following file names:</p> <table style="width: 100%; border: none;"> <tr> <td style="padding-left: 40px;">MLB Pipette Inventory</td> <td style="text-align: right;">QC-19-09_F1.xlsx</td> </tr> <tr> <td style="padding-left: 40px;">Pipette Verification Record Sheet</td> <td style="text-align: right;">QC-19-09_F2.xlsx</td> </tr> <tr> <td style="padding-left: 40px;">Pipette Verification – Gravimetric Analysis Form</td> <td style="text-align: right;">QC-19-09_F3.xlsx</td> </tr> <tr> <td style="padding-left: 40px;">Pipette Verification – Gravimetric Analysis Spreadsheet</td> <td style="text-align: right;">QC-19-09_F4.xlsx</td> </tr> </table>	MLB Pipette Inventory	QC-19-09_F1.xlsx	Pipette Verification Record Sheet	QC-19-09_F2.xlsx	Pipette Verification – Gravimetric Analysis Form	QC-19-09_F3.xlsx	Pipette Verification – Gravimetric Analysis Spreadsheet	QC-19-09_F4.xlsx
MLB Pipette Inventory	QC-19-09_F1.xlsx								
Pipette Verification Record Sheet	QC-19-09_F2.xlsx								
Pipette Verification – Gravimetric Analysis Form	QC-19-09_F3.xlsx								
Pipette Verification – Gravimetric Analysis Spreadsheet	QC-19-09_F4.xlsx								
15. References	1. CRC Handbook of Chemistry and Physics. 93 rd ed. CRC Press: Boca Raton, FL, 2012; p 6-8.								

Sample Pipette Verification Record Sheet
 OPP Microbiology Laboratory

Verification Date(s): _____ Initials: _____

<i>Manufacturer</i>	<i>Model No.</i>	<i>Serial No(s).</i>	<i>Volume Range</i>	<i>In-house Verification Volume(s)</i>	<i>Verification Status¹</i>	<i>Corrective Action (Y/N)</i>
Gilson	M10	X12523D	1-10 µL	10 µL		
		X12649D				
		BH15232				
		MH05165				
Gilson	M100	GG05125	10-100 µL	100 µL		
		GG05127				
Gilson	Distribiman	AE10020	1-1250 µL	10 µL (125 µL tip) 100 µL (1250 µL tip) 900 µL (12.5 mL tip)		
		BD10010				
		U10048H				
Eppendorf	Repeater M4	H35817G	20 µL- 10 mL	30 µL (1 mL tip) 100 µL (5 mL tip) 900 µL (10 mL tip) 10 mL (50 mL tip)		
Rainin	L-1000	A0504243A	100- 1000 µL	1000 µL		
		L0508039A				
		C0823986A				
		J0753884A				
		C0825980A				
		C0823596A				
		H0101474A				
		H0100977A				
		J0902334A				
		J0908624A				
		D0303509A				
E0301364A						
Rainin	L-200	A0510192A	20-200 µL	100 µL		
		L0509218A				
		C0821962A				
		J0750805A				
		C0820542A				
		C0820661A				

<i>Manufacturer</i>	<i>Model No.</i>	<i>Serial No(s).</i>	<i>Volume Range</i>	<i>In-house Verification Volume(s)</i>	<i>Verification Status¹</i>	<i>Corrective Action (Y/N)</i>
		G0101809A				
		G0102379A				
		J0902612A				
		C0401654A				
Rainin	L-100	C0825210A	10-100 µL	100 µL		
		C0825238A				
Rainin	L-20	A0507382A	2-20 µL	10 µL		
		C0822081A				
		J0724932A				
		C0822312A				
		C0825315A				
		F0100492A				
		F0100448A				
		J0903005A				
		C0400398A				
Rainin	L-2	H0100116A	0.1-2 µL	2 µL		
		H0100003A				
Rainin	L-5000	D1080497A	0.5-5 mL	5 mL		
Rainin	L-10000	A1058487A	1-10 mL	10 mL		
Rainin	L-20000	L0931886A	2-20 mL	20 mL		

¹Verification status = PASS if percent inaccuracy from gravimetric analysis is within ±5% or if vendor calibration certificate indicates acceptable results. If verification status = FAIL, record “yes” in Corrective Action column and fill in *Action Taken* below.

Corrective Actions:

Pipette Serial Number

Action Taken (QAO must be notified)

1.) _____

2.) _____

3.) _____

Sample Pipette Inventory
 OPP Microbiology Laboratory

MLB Pipette Inventory

<i>Manufacturer</i>	<i>Model No.</i>	<i>Serial No(s).</i>	<i>Manufacturer</i>	<i>Model No.</i>	<i>Serial No(s).</i>
Gilson	M10	X12523D X12649D BH15232 MH05165	Rainin	L-200	A0510192A L0509218A C0821962A J0750805A C0820542A C0820661A G0101809A G0102379A J0902612A C0401654A E0615366A
Gilson	M100	GG05127 GG05125			
Gilson	Distri-man	AE10020 LD05524 U10048H			
Eppendorf	Repeater M4	H35817G			
Rainin	L-1000	A0504243A L0508039A C0823986A J0753884A C0825980A C0823596A H0101474A H0100977A J0902334A J0908624A D0303509A E0301364A E0613583A	Rainin	L-100	C0825210A C0825238A
		Rainin	L-20	A0507382A C0822081A J0724932A C0822312A C0825315A F0100429A F0100448A J0903005A C0400398A	
		Rainin	L-2	H0100116A H0100003A	
		Rainin	L-5000	D1080497A	
		Rainin	L-10000	A1058487A	
		Rainin	L-20000	L0931886A	
		Rainin	L-10 (8 ch)	B1102294A	
		Rainin	L-20 (8 ch)	F0300384A	
		Rainin	L-200 (8 ch)	H0103189A	
		Rainin	L-200 (12 ch)	H0102273A	

Updated: 02/15/18