US Environmental Protection Agency
Office of Pesticide Programs

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

Verification of Digital Pipettes

SOP Number: QC-19-11

Date Revised: 02-15-22

SOP Number	QC-19-11
Title	Verification of Digital Pipettes
Revisions Made	• Minor editorial changes for clarification purposes. • Clarifications added to Section 9, Data Management.

SOP Number	QC-19-11
Title	Verification of Digital Pipettes
Scope	Describes the process for verification of digital pipettes.
Application	Pipettes are calibrated by an ISO 17025 accredited vendor at a frequency determined by the laboratory and are verified in-house using the gravimetric procedure as necessary.

	Approval	Date
SOP Developer:	Rebecca Pines Print Name: Rebecca Pines	02/18/2022
SOP Reviewer	Laura Burton Print Name: Laura Burton	02/18/2022
Quality Assurance Unit	Kiran Verma Print Name: Kiran Verma	02/18/2022
Branch Chief	Rebecca Pines Print Name: Rebecca Pines (Acting Branch Chief)	02/18/2022

Date SOP issued:	02/18/2022
Controlled copy number:	0
Date SOP withdrawn:	

TABLE OF CONTENTS

1. Definitions	Abbreviations/definitions are provided in the text. 1. Verification failure = Verification of pipette exceeds ±5% inaccuracy and/or <5% of the coefficient of variation (% CV). 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). 3. Percent inaccuracy = percentage by which measured volume differs from the target volume
2. Health and Safety	Follow procedures specified in SOP MB-01, Laboratory Biosafety.
3. Personnel Qualifications and Training	Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
4. Instrument Calibration	1. The pipets are calibrated by an ISO 17025 accredited vendor or verified in-house (refer to section 12.2) on a predetermined frequency (see the Pipette Verification Record Sheet, section 14). 2. 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	Pipettes with inaccuracies that exceed the specifications listed in this SOP (see section 8.2); remove these pipettes from use until brought back into specifications.
8. Non-conforming Data	1. Manage non-conforming data consistent with SOP ADM-07, Non-Conformance Reports. 2. Do not use pipettes if the inaccuracy exceeds ±5% of the target volume and/or <5% CV (see 13.1). For corrective actions, see section 12.3.
9. Data Management	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 electronic inventory, file a hard copy of the current pipette inventory in the Pipette Verification and Calibration Record Book and maintain all previous copies of the inventory.

10. Cautions	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 Materials	1. *Calibrated balances.* Capable of measuring 0.01 g for verifying pipettes with volumes greater than 1 mL and 0.0001 g for verifying pipettes with volumes less than or equal to 1 mL. a. For balances capable of measuring up to 0.00001 g, use the upper weight range of the balance in order to display a resolution of 0.0001 g prior to initiating gravimetric pipette verification procedure. 2. *Pipettes.* b. Rainin Adjustable Volume Pipettes c. Gilson Microman Positive Displacement Pipettes d. Gilson Distriman Continuously Adjustable Volume Repetitive Pipettes e. Eppendorf Repeater Pipettes
12. Procedure and Analysis	
12.1 Pipette Verification Requirements	a. Verify and service pipettes using an ISO 17025 accredited vendor; refer to the Pipette Verification Record Sheet (see section 14) for the frequency of verification. b. If a pipette is dropped or broken, verify the pipette using the gravimetric verification procedure (see section 12.2). c. Record vendor or in-house verification 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.
12.2 Gravimetric Verification Procedure	The gravimetric pipette verification procedure utilizes the relationship between the weight of a water sample (g) and its density (g/mL) to verify the volume being delivered at a specified temperature. a. Use the pipette tips that correspond to each pipette. b. Record all pertinent information for the gravimetric verification procedure on the Pipette Verification – Gravimetric Analysis Form (refer to section 14). c. In advance of testing, fill a container with de-ionized water and allow it to equilibrate to room temperature in the same laboratory as the

	balance. Record the balance used on the appropriate form. d. Place a small container on the balance (for example, a 50 mL Erlenmyer flask) and record its weight on the appropriate form. e. Using the pipette to be verified, aspirate an aliquot of DI water from the sample aliquot container and dispense into the small container. Refer to the Pipette Verification – Gravimetric Analysis Spreadsheet (see section 14) for the recommended verification volume for each pipette. f. Record the weight on the appropriate form. g. 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 weight on the appropriate form. h. Input the measurements and other appropriate information into the pipette-specific worksheet in the Pipette Verification – Gravimetric Analysis Spreadsheet (see section 14). i. Verify that the percent inaccuracy is within ±5% and the % CV is <5%. i. If either the percent inaccuracy or % CV are outside the required range, repeat the analysis. ii. If the ranges continue to be outside the required range, label the pipette as "Out of Service" and remove the pipette from use until it is repaired and subsequently successfully verified.
12.3 Pipette Verification Record Sheet	a. From the data on the calibration certificate or Pipette Verification – Gravimetric Analysis Spreadsheet for each pipette, record the following on the Pipette Verification Record Sheet: i. *Verification Status*: record as "pass" or "fail." Record status as "pass" if the percent inaccuracy is within ±5% and the % CV is <5% from gravimetric analysis or if vendor calibration certificate indicates acceptable results, otherwise record as "fail." ii. *Corrective Action*: 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.

	b. Complete the Pipette Verification Record Sheet electronically or by hand.
13. Data Analysis/ Calculations	1. $Percent\ Inaccuracy = \frac{(\bar{x} - ideal\ volume) \times 100}{ideal\ volume}$ 2. $\%\ CV = \left(\frac{Standard\ deviation}{Mean}\right) \times 100$
14. Forms and Data Sheets	Test Sheets. Test sheets are stored separately from the SOP under the following file names: MLB Pipette Inventory — QC-19-11_F1.xlsx Pipette Verification Record Sheet — QC-19-11_F2.xlsx Pipette Verification – Gravimetric Analysis Form — QC-19-11_F3.xlsx Pipette Verification – Gravimetric Analysis Spreadsheet — QC-19-11_F4.xlsx
15. References	CRC Handbook of Chemistry and Physics. 93rd ed. CRC Press: Boca Raton, FL, 2012; p 6-8.