



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 Using
the PCS 2 Pipette Calibration System**

SOP Number: QC-19-04

Date Revised: 07-01-08

EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

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Initiated By: _____ Date: ___/___/___

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1.0 SCOPE AND APPLICATION:

1.1 The Artel PCS 2 pipette calibration system quickly and accurately measures the actual volume dispensed by any pipette and will be used by the laboratory to verify pipette performance. Results are traceable to NIST.

2.0 DEFINITIONS:

2.1 NIST = National Institute of Standards and Technology

2.2 Verification failure = Verification of pipette exceeds $\pm 5\%$ of the target volume.

3.0 HEALTH AND SAFETY:

3.1 All kit reagents are non-hazardous, aqueous based and may be disposed of by flushing down a drain.

4.0 CAUTIONS:

4.1 Make sure the PCS Instrument is kept out of direct sunlight and away from anything hot or cold. The pipettes, the instrument, and the reagent kit should be stored at room temperature.

4.2 To ensure proper sample mixing, place the PCS Instrument on a sturdy table.

4.3 Do not open the PCS Vial Holder Cover until you are prompted with "Pipette Sample #" or "Remove Cap and Insert Blank."

4.4 If a pipette is dropped or damaged, it must be successfully verified prior to use.

4.5 If a pipette fails verification, it will not be used in the laboratory and will be recalibrated prior to use.

5.0 INTERFERENCES: Issues discussed in 5.1 through 5.8 must be recognized to avoid interference during the verification process. Interference could lead to erroneous readings.

5.1 Ensure that the reagent kit is at the same ambient temperature as the instrument by storing them in close proximity to each other (in the same laboratory at room temperature).

5.2 Once the blank reagent vial has been inserted into the instrument it must not be turned, moved, or removed until the entire procedure has been completed, or the vial has been consumed. If the vial is removed and sample solution has been

pipetted into the vial, this vial is no longer usable.

- 5.3 Blank reagent vials should be moved immediately from the vial block into the vial holder, giving them no chance to warm or cool during the verification process.
- 5.4 Always take a fresh aliquot of sample solution if the solution has been in the sample aliquot container for over one-half hour.
- 5.5 Always place the cap (lightly) on the aliquot containers between pipette sampling to minimize reagent evaporation.
- 5.6 The PCS reagent kit should be stored at room temperature. Avoid storage temperatures above 32EC (90EF). Kit components should be kept in the dark.
- 5.7 To ensure that accurate and precise results are obtained during pipette verifications and instrument calibration checks, all vials should be free of fingerprints or smudges prior to placement into the vial block. **THUS, AVOID HANDLING THE LOWER PORTION OF THE VIALS.** Fingerprints or smudges on the vials that fall into the optical path will introduce errors that cause inaccurate readings. In the case of instrument calibration checks, false failures may occur. All vials are cleaned and inspected prior to shipping. If the lower portion of the vial is inadvertently touched, it should be properly cleaned prior to use. The following procedure is recommended for cleaning all PCS vials:
 - 5.7.1 Obtain rubbing alcohol (isopropyl) and pour into a small container. Pour enough rubbing alcohol into the container so as to cover approximately 1 inch of the vial when immersed in the alcohol.
 - 5.7.2 Holding the vial by the cap, immerse (approximately 1 inch) in the alcohol.
 - 5.7.3 Remove the vial from the alcohol and, using a lint free wipe (e.g., Kimwipe), wipe the vial dry by rotating the vial in the lint free wipe several times.
- 5.8 The power line to which the instrument is connected should be free of voltage variations caused by other equipment such as water baths or refrigerators.

6.0 PERSONNEL QUALIFICATIONS:

- 6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the

training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

- 7.1 ARTEL PCS 2 Pipette Calibration System Instrument, serial no. 6250
- 7.2 Rainin Adjustable Volume Pipettes
- 7.3 Gilson Microman Positive Displacement Pipettes
- 7.4 Gilson Distriman Continuously Adjustable Volume Repetitive Pipette

8.0 INSTRUMENT OR METHOD CALIBRATION:

- 8.1 The PCS instrument calibration should be checked monthly. To check the instrument calibration, an instrument calibrator kit is used. It contains four vials of different concentrations of mixed sample and blank reagents and an insert with calibrator values, lot code, expiration date and instructions. The calibrator values contain information about these concentrations derived from a reference instrument at the factory which itself has been calibrated using standards traceable to NIST.
- 8.2 The PCS instrument calibration check printout will be copied (it is printed on thermal paper which fades over time) and stored in the Pipette Verification and Calibration Log Book.
- 8.3 Certificates of calibration for PCS calibrator kit and PCS Reagent Kit are stored in the Pipette Verification and Calibration Log Book.
- 8.4 Refer to Chapter 5 of the PCS Procedure Guide for detailed instructions and pass/fail specifications.

9.0 SAMPLE HANDLING AND STORAGE: Between uses, the calibration kit should be stored in darkness at room temperature (18-28EC) to allow for a six month shelf life.

10.0 PROCEDURE AND ANALYSIS:

- 10.1 Pipette verification will be performed quarterly on the micropipettes listed in Attachment A using the Artel PCS 2 pipette calibration system (refer to reference 15.2). The results are recorded on the Pipette Verification Record Sheet (refer to

section 16.0).

10.2 All pipettes will be calibrated annually by a vendor.

NOTE: The annual vendor calibration will serve as pipette verification for that quarter.

10.3 Turn on the PCS instrument and the printer.

10.4 Blank vials should be loaded into the vial block at least 15 minutes prior to their use. Four vials can be loaded into the vial block at this time. Inspect for smudges and clean them if necessary (refer to section 5.7).

10.5 Allow the instrument to self calibrate and follow the on-screen instructions. Enter a pre-assigned Operator ID number.

10.6 When prompted, enter the four or five digit lot code shown on the reagent kit insert and on the exterior label affixed to the top of the reagent kit. Enter the lot information if it is a new lot of reagents.

10.7 After self calibration, the instrument will prompt the operator to insert the CAL A Vial.

10.7.1 **IMPORTANT:** The CAL A calibration standard should always be inserted into the vial holder with the CAL A label facing toward the front of the PCS. Be careful not to smudge or get fingerprints on the lower portion of the vial as this is a part of the optical path and any smudges will decrease the accuracy of the results (refer to section 5.7). Never remove the cap of a calibration standard.

10.8 After closing the cover on the vial holder, the PCS will perform a zero check. Once the zero check is complete, remove the CAL A vial and place it back in the Reagent Kit.

10.9 Remove a blank vial from the block. Invert it several times to mix the contents thoroughly, remove the cap and set it aside.

10.9.1 **IMPORTANT:** Each blank reagent vial must be handled with care. The vial contains a predetermined amount of liquid and any spillage will lead to inaccurate results. The lower portion of the vial is a part of the optical path and smudges will decrease the accuracy of the results. Touching it should be avoided (refer to section 5.7).

10.10 Open the vial holder cover and insert the blank reagent vial into the vial holder located under the vial holder cover. Close the cover. The instrument will take a reading of the blank.

10.10.1 **IMPORTANT:** Once the blank reagent vial has been inserted into the instrument it must not be turned, moved or removed until the verification procedure is complete for all pipettes to be verified using that vial.

10.11 Enter the pipette serial number. Enter the volume to be checked. Once entered, the instrument will indicate which range solution should be used for that verification.

10.11.1 Refer to “Verification Volume” column in Attachment A for the pipette volumes to be verified.

10.12 Locate the appropriate range solution in the reagent kit. Mix the contents by inverting the bottle several times before removing the cap. Recap the bottle as soon as possible to avoid evaporation.

10.12.1 **IMPORTANT:** Each kit contains bottles of sample solution corresponding to four different pipette volume ranges:

Range 1: For pipette volumes 200 to 5000 μL .

Range 2: For pipette volumes 50 to 199 μL .

Range 3: For pipette volumes 10 to 49 μL .

Range 4: For pipette volumes 2 to 9 μL .

YOU MUST USE THE PROPER RANGE OF SOLUTION FOR THE PIPETTE VOLUME YOU ARE VERIFYING. If the improper sample solution has been pipetted into a blank reagent vial, that vial is no longer usable and should be discarded.

10.13 Transfer an aliquot of sample solution, using a disposable transfer pipette, into a sample aliquot container. Transfer pipettes and sample aliquot containers are found in the reagent kit. Place the container into the aliquot container holder to avoid any possibility of spillage. Place the cap lightly on the container to minimize evaporation.

10.13.1 **NOTE:** Transfer only enough reagent to be completely used in half-hour increments. The total volume of sample solution required depends on the pipette volume, the total number of pipettes tested and

the total number of discrete data points being generated. The volume graduations on the pipette as well as the 1 mL marks on the sample aliquot containers can be used as rough guide.

- 10.14 Using the pipette to be verified, aspirate an aliquot of sample solution from the sample aliquot container. Use your standard pipetting technique. Place the cap lightly back on the sample aliquot container between sampling to avoid errors due to reagent evaporation.
- 10.14.1 NOTE: When verifying the accuracy of a pipette there is an inevitable linkage between the operator and pipette. For meaningful results, it is recommended that you use your standard laboratory pipetting technique and follow your specific pipette manufacturer's instructions.
- 10.15 Lift the instrument vial holder cover and dispense the aliquot of sample solution into the blank reagent vial.
- 10.15.1 IMPORTANT: The sample solution may be dispensed directly into the liquid or against the inside wall of the vial. If dispensing against the inside wall, the pipette tip should be placed above the meniscus but below the top of the vial holder. The mixing action of the PCS will wash the solution off the inside wall of the vial.
- 10.16 Once the aliquot is dispensed, carefully withdraw your pipette and close the cover. The PCS will display the results of the previous reading and will prompt you to add the next sample. Follow the same above procedure for sample #2 and for each subsequent sample addition. After each new sample addition, the instrument will mix, read, and print the delivered volume.
- 10.17 When you have dispensed the desired number of samples for the verification run (5 samples, refer to Attachment A), press the "End of Run" button. The group statistics will be computed and the results printed.
- 10.18 Within a blank vial's allowed capacity, you may continue with additional verification runs. You may perform verifications all at the same sample volume, at lower or higher sample volumes but within the same range solution, or at lower or higher sample volumes and with different range solutions.
- 10.18.1 IMPORTANT: In verifying pipettes from more than one range in a given vial, always use the range of sample solution which is appropriate to the volume of pipette being verified.

10.19 In verifying an adjustable volume pipette at multiple volumes, you may start anywhere along its volume range. When you have dispensed the desired number of samples at the first volume, press “Enter” (do not press “End of Run”). The group statistics for the first volume will be computed and the results printed. Follow the on screen instructions and indicate the next volume to be verified when prompted. The instrument knows that you are verifying the same pipette but at a different volume. You may continue to verify the same pipette at different volumes by pressing the “Enter” key at the conclusion of each volume. Be sure to pay strict attention to the range solution to be used for a particular volume. After pipetting the final sample at the last volume for the pipette press the “End of Run” button. The group statistics and results for the last volume and the footer will be printed.

11.0 DATA ANALYSIS/CALCULATIONS: None.

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data will be recorded promptly, legibly, and in indelible ink on the appropriate forms. Completed forms are archived in notebooks kept in secured file cabinets in file room D217. Only authorized personnel have access to the secured files. Archived data is subject to OPP=s official retention schedule contained in SOP ADM-03, Records and Archives.

12.2 An inventory of pipettes will be maintained electronically using a Microsoft Excel spreadsheet (refer to 16.3). After each addition to or deletion from the inventory, a hard copy of the pipette inventory will be filed in the Pipette Verification Record Book.

13.0 QUALITY CONTROL:

13.1 For quality control purposes, the required information is documented on the appropriate forms (refer to section 16.0).

13.2 To provide maximum life and dependable system performance, periodic cleaning should be performed. Refer to Chapter 7, Section 7.3, of the PCS Procedure Guide for instructions on cleaning the cabinet, the vial holder, and the filer wheel assembly.

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 Acceptable limits for PCS Instrument Calibration are listed in the PCS 2 Pipette Calibration System Procedure Guide (refer to reference 15.1). If Noise,

Accuracy, Inaccuracy Due to Noise, Overall System Inaccuracy, or Overall Instrument Performance failure, clean the calibration vials and perform the instrument calibration check again. If the instrument fails again, purchase another Calibration set and recheck the calibration. If failures persist, schedule service for the instrument. Do not use the instrument for calibration until it has been recertified.

- 14.2 Do not use pipettes if the inaccuracy exceeds $\pm 5\%$ of the target volume.
- 14.3 See Chapter 7, Troubleshooting, of the PCS Procedure Guide for specific system performance checks. Section 7.2 provides a troubleshooting guide for symptoms, causes, and corrective actions.

15.0 REFERENCES:

- 15.1 Artel, Inc. 1997. PCS 2 Pipette Calibration System Procedure Guide, Doc #: 15A2135 Ver: 5.1, 03/28/97.
- 15.2 Artel, Inc. 2003. Artel Lab Report – Calibration Frequency for Pipettes, Issue 6.

16.0 FORMS AND DATA SHEETS:

- 16.1 Pipette Calibration Record Sheet
- 16.2 Sample PCS Printout
- 16.3 Sample Pipette Inventory
MS Excel spreadsheet: MLB Pipette Inventory

Pipette Verification Record Sheet
 OPP Microbiology Laboratory

PIPETTE VERIFICATION SUMMARY			
Date		Volume Tested	
Initials		Range Solution	
Pipette Vol. Range		Replicates	
Serial No.		Reagent Lot No.	
Sample #	Volume	Mean	
1		Standard Dev.	
2		%CV	
3		Inaccuracy	
4		Pass/Fail	
5		Nonconformance	
Comments:			

PIPETTE VERIFICATION SUMMARY			
Date		Volume Tested	
Initials		Range Solution	
Pipette Vol. Range		Replicates	
Serial No.		Reagent Lot No.	
Sample #	Volume	Mean	
1		Standard Dev.	
2		%CV	
3		Inaccuracy	
4		Pass/Fail	
5		Nonconformance	
Comments:			

Sample PCS Printout
OPP Microbiology Laboratory

US EPA / MARYLAND
FT. MEAD, MD 20755

DATE: 00/00/00
TIME: 00:00

PIPETTE CALIBRATION

PIPETTE SERIAL #: 152348
OPERATOR ID: 1
REAGENT LOT #: 14758
TEMPERATURE (C): 22.1

PIPETTE SAMPLE VOLUME: 10.00 μ L
SAMPLE RANGE: 3

SAMPLE #	VOLUME
1	9.94
2	9.97
3	9.94
4	10.03
5	9.98

NO.PTS: 5
MEAN: 9.97
STD: 0.037
%CV: 0.37%
INACC: -0.28%

LAST INST. CALIBRATION CHECK: 27 Oct 00
SOFTWARE VERSION: 6.013
INSTRUMENT SERIAL #: 6250
ARTEL PCS , PATENTS PENDING

Sample Pipette Inventory
 OPP Microbiology Laboratory

MLB Pipette Inventory						
<i>Manufacturer</i>	<i>Model No.</i>	<i>Serial No(s).</i>	<i>Volume Range</i>	<i>Verification Volume(s)</i>	<i>Replicates</i>	<i>Verification Interval</i>
Gilson	M10	X12523D X12649D BH15232	0.5-10 µL	10 µL	5	3 months
Gilson	Distriman	AE10020	1-12.5 µL ¹ 10-125 µL ² 100-1250 µL ³	10 µL ¹ 100 µL ² 900 µL ³	5	3 months
Rainin	L-1000	A0504243A L0508039A C0823986A J0753884A	100-1000 µL	1000 µL	5	3 months
Rainin	L-200	A0510192A L0509218A C0821962A J0750805A	20-200 µL	100 µL	5	3 months
Rainin	L-20	A0507382A C0822081A J0724932A	2-20 µL	10 µL	5	3 months

¹125 µL syringe: 1-12.5 µL volume range

²1250 µL syringe: 10-125 µL volume range.

³12.5 mL syringe: 100-1250 µL volume range