Standard Operating Procedure for the PCS® Pipette Calibration System

Prepared by ARTEL
based on CLSI approved guidelines
March 2007

(For use with firmware versions 7A2.XXX, where" XXX"
represents a three digit number between 001 and 200)

Company _____ **Procedure #:** _____

Procedure Title	Operation of the PCS® Pipette Calibration System

Prepared by	Effective date	Supersedes Procedure #

Review Date	Revision Date	Signature

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Company _____ **Procedure #:** _____

PRINCIPLE:

Accurate volumetric measurements and transfers are essential to all laboratories concerned with maintaining high levels of quality and productivity. The performance of pipettes varies substantially, and their accuracy cannot be taken for granted.

The PCS® Pipette Calibration System uses a high performance photometer and proprietary reagents to quickly and accurately measure the volume dispensed by any pipette. The PCS delivers accuracy and precision at pipette volumes from 0.1 µL to 5000 µL.

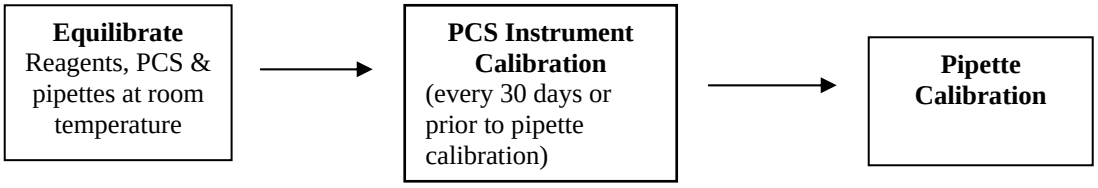
A PCS user can perform a ten-sample pipette calibration in less than three minutes. After each sampling, the PCS will calculate and display the volume of liquid delivered. A printout is generated displaying the volume of each sample and group statistics. To assist with effective record-keeping, the printout also shows pipette Serial number, operator, time and date, etc. Test results are traceable to NIST (National Institute of Standards and Technology).

Additionally, the PCS is effective for improving pipetting technique. Feedback is immediate and the system is used easily with minimal training. Pipette performance can be verified when needed, where needed, and by personnel who actually use the pipettes, helping to ensure smooth workflow and optimum quality and efficiency in the laboratory.

Calibration checks on new pipettes require a minimum of thirty data points. For pipettes already in service, calibration checks are to be performed quarterly using at least ten data points for each calibration run. Quick checks can be performed monthly using at least four data points.

PROCESS FLOW CHART:

Preparation → **Instrument Check** → **Pipette Calibration**



REQUIRED EQUIPMENT AND MATERIALS:

Equipment:

1. PCS Instrument with power supply
2. DPU 414 Printer with AC adaptor
3. Printer cable
4. Printer paper
5. Aliquot Container Holder
6. Pipettes to be calibrated

Materials:

1. Exhibit A; PCS Instrument & Accessories Schematic
2. PCS Reagent Kit, containing the following:
   - Blank Reagent Vials
   - Sample Solutions
   - Calibration Standard
   - Kit Insert
   - Sample Aliquot Containers (optional in Bulk Reagent Kit)
   - Transfer Pipettes (optional in Bulk Reagent Kit)
3. PCS Instrument Calibrator Kit containing the following:
   - CAL A vial
   - CAL B vial
   - CAL C vial
   - CAL D vial
   - Kit Insert
4. Pipette tips appropriate for pipettes being calibrated

Preparation:

The pipette to be checked should be clean and well maintained. All reagents and pipettes should be allowed to equilibrate to the same ambient temperature before use.

All vials should be free of fingerprints or smudges prior to placement in the Vial Block to equilibrate. To clean vials, hold by the cap and immerse the lower portion (about 2 cm) of the vial into a container of clean, HPLC grade isopropyl alcohol. Remove the vial from the alcohol and gently wipe the lower portion of the vial by rotating the vial against a lint-free wipe.

All Vials should be placed in the Vial Block for fifteen (15) minutes prior to use to allow them time to equilibrate to the temperature of the instrument.

Storage Requirements:

The PCS can be left on for an indefinite period. The photometer lamp used in sample measurement is on only during measurement. The life expectancy of the lamp is not adversely affected by leaving the instrument on continuously.

PCS Reagents should be stored in their original kit box at room temperature (18°C to 28°C). Any Sample Solution dispensed into an aliquot container should be discarded. To avoid contamination, do not return reagents to their original bottle after use.

The PCS Instrument Calibrator Kit should be stored in darkness (closed box) and at room temperature (18°C to 28°C) between uses to ensure the nine-month shelf life. The contents of the kit are subject to degradation with time, temperature and exposure to light.

INSTRUMENT ZERO AND CALIBRATION CHECK:

When in use, the PCS Instrument should be zeroed daily according to the procedure outlined in this section. Prior to use, the Reagent Kit Cal A Calibration Standard should be allowed to equilibrate in the Vial Block for at least 15 minutes.

At a minimum, the PCS Instrument calibration should be checked every thirty days or prior to next use, whichever is later. The Instrument calibration is checked using the Instrument Calibrator Kit.

Procedures:

1. Instrument Zero

   The CAL A Calibration Standard from the Reagent Kit is used to zero the PCS. Since a small amount of drift will occur due to temperature variations, it is important to compensate for this effect by using the CAL A Calibration Standard specific for the Reagent lot in use. During operation, the Instrument will require a Zero Check under three conditions: 1) prior to the first pipette calibration after power on; 2) more than 8 hours has elapsed since the last Zero Check and 3) if a change in temperature of ±2°C has occurred since the last Zero Check.

   The prompt "Insert CAL A" will be displayed. Remove the Reagent Kit CAL A from the vial block and insert it into the vial holder with the CAL A label facing the front of the instrument. Press down until the vial reaches the bottom of the vial holder. Close the cover. When the instrument is finished performing the Zero Check, the prompt "Please Remove Vial" will appear. Remove the vial and continue with operation.

2. Complete PCS Instrument Calibration Check

   The PCS Instrument Calibrator Kit contains four vials (CAL A, B, C and D) with calibrator values, Lot Code, and expiration date which are located on the label on the inside of the box lid. The calibrator values communicate reference information about the concentrations derived from a factory reference instrument that is calibrated using NIST traceable standards.

Preparation:

Place clean PCS Instrument Calibrator Kit vials into the Vial Block. Prior to use, the vials should be allowed to equilibrate in the Vial Block for at least 15 minutes.

Never remove the cap of any Calibrator vial (this includes the CAL A that is part of the Reagent Kit). Removal of the cap will affect the concentrations of the solutions and could adversely affect results of the instrument calibration. The label is placed on the vial so that the "CAL -" should always face the front of the instrument.
To access the Instrument Calibration from the Main Menu, respond to the Instrument's prompts as follows:

PROMPT	**RESPONSE**
"Pipette Calibration?"	NO or MENU SCROLL
"Instr. Calibration?"	YES
"Instrument Cal. Menu	
Perform Instr. Cal?	YES

The instrument will then check the temperature and, if it is outside the specified range for proper operation (18°C to 28°C), the following prompt will be displayed", "Temp out of Range; Continue (Y/N)". If NO is pressed, the Main Menu will be displayed. If YES is pressed, the operator may continue with the Instrument Calibration. Note: There is no certainty that the obtained PCS results will meet the system specifications.

INSTRUMENT CALIBRATION

Procedures: continued

At the following prompt, "Enter Operator ID: ", enter a number (not exceeding 20 characters) identifying the operator for this Instrument Calibration. Alphabetic characters may be used by pressing the MENU SCROLL, choosing the letter and then pressing ENTER for each character. The display will then prompt the user to enter the Calibrator Lot Code. The Calibrator Lot Code is found on the inside of the box lid in the Calibrator Kit. It will be a four or five digit number. After entering the Calibrator Code, press ENTER. If this Lot Code matches the one previously entered, the sequence will proceed.

If the Lot Codes do not match, the display will prompt "Cal Lot XXXXX New Lot? (Y/N)". If the lot is not new, enter NO and reenter the correct Calibrator Lot Code. If the lot is new, answer YES. The new calibrator values now need to be entered. These values are found on the insert card. The calibrator values consist of three five-digit numbers. Each group is entered separately by pressing enter between each group. These will be followed by the expiration date. Enter the month and year of the expiration date as a four digit number (mmyy). All leading zeroes and decimal points must be entered, including any leading zero that may occur in the expiration date. The Instrument will now display "Lot Code: XXXXX Correct? (Y/N)". Press YES to confirm and continue with the Instrument Calibration.

After the Calibrator Lot Code is successfully entered, the Instrument will take a series of dark readings and then prompt: "Insert Cal A Vial". Leaving the cap in place, place the CAL A vial into the instrument with the label facing the front of the instrument. When the readings are completed, the Instrument will prompt: "Please Remove Vial". When the vial is removed, the prompt will read: "Insert CAL B Vial". CAL B, CAL C, and CAL D are sequentially inserted, following the prompts. The entire process requires about twelve minutes to complete.

When the Instrument Calibration Check is complete, the results will be printed out. Near the bottom of the printout, the OVERALL CALIBRATION RESULT will be shown with either Pass or Fail. This indicates whether the instrument has successfully passed the calibration check. If the instrument fails the calibration check, refer to the Troubleshooting Guide in section 7.2 of the PCS Procedure Guide for instructions on how to proceed. If the instrument passes the calibration check, the date of the Last Instrument Calibration Check will change to the current day. If the Instrument Calibration fails, the date will not change. Retain the printout as documentation.

PIPETTE CALIBRATION:

Instrument Preparation:

1. Turn on the Instrument and the Printer. Be sure the Printer is ON LINE.

2. Before performing this Procedure, ensure that the Reagent Kit is at the same temperature as the Instrument.

3. Upon power-on, the Instrument will perform a system test.

4. When the system test is complete, a pipette calibration can be initiated.

PIPETTE CALIBRATION: continued

Pipette Calibration Check:

1. Check the display to be sure it reads "Main Menu, Pipette Calibration?". If it does not, press RESET to return to this display.

2. When the display reads "Main Menu, Pipette Calibration?" press YES to begin the pipette calibration procedure. The Instrument will then check the temperature and, if it is outside the specified range for proper operation (18°C to 28°C), the following prompt will be displayed: “Temp out of range Continue? (Y/N)”. If NO is pressed, the Main Menu will be displayed. If YES is pressed, the operator may continue with the calibration, but there is no certainty that the results the PCS obtains will meet the system specifications.

3. The Instrument will then display "Enter Reagent Lot Code:__". Enter the Lot Code. The Lot Code is shown on the Reagent Vials, the Reagent Kit insert and on the exterior of the Reagent Kit box. The Instrument maintains a library of up to 6 previously entered Lot Codes. If this Lot Code matches one previously entered, the sequence will proceed.

4. If the Lot Code does not match, the display will read "Rgt Lot Code XXXXX New Lot (Y/N)?". If this is not a new lot of reagent, press NO and reenter the correct Lot Code. If this is a new lot, press YES. Enter the Lot Code and Group Numbers found on the correct side of the Lot Code Card found inside the Reagent Kit box. Press Enter after entry of each group of digits. At the next prompt, enter the month and year of the expiration date of the Reagent Kit as a four digit number (mmyy).

5. When finished, the display will read "Lot Code: XXXXX Correct? (Y/N)". Enter YES to confirm.

6. The Instrument will perform a brief self-calibration, followed by: “Insert Cal A Vial”. Insert the Cal A vial that is included in the Reagent Kit with the front of the label facing forward. Follow the prompts to close the cover and remove the vial.

7. Once the Instrument Zero Check is complete, the display will read: "Remove Cap and Insert Blank".

8. Remove a Blank Vial from the vial block. The Blank Reagent is light blue in color.

   NOTE: Each Blank Reagent Vial must be handled with care. First, it contains a predetermined amount of liquid and any spillage will reduce accuracy. Second, the lower portion of the Blank Vial is a part of the optical path and smudges will decrease the accuracy of the results. Touching this region should be avoided. Third, to ensure a consistent optical path, once the Blank Reagent Vial has been inserted into the Instrument, it must not be turned, moved or removed until the calibration procedure is completed for all pipettes to be calibrated using that vial.

9. Remove the cap, open the Vial Holder Cover and insert the Blank Reagent Vial into the Vial Holder, pressing it down until it contacts the bottom of the vial holder. Close the cover.

10. After the blank reading has been obtained, the display will read: "Enter Operator ID". Enter a number (not exceeding 20 digits) identifying the operator for this calibration. This number appears on the Printout. Alphabetic characters may be used by pressing the MENU SCROLL, choosing the letter and then pressing ENTER for each character.

PIPETTE CALIBRATION:

Pipette Calibration Check: continued

11. The next prompt will read "Enter Pipette ID: _____ ". Enter the pipette ID (not exceeding 20 digits) and press Enter. This ID will be appear on the Printout for pipette cross-referencing, and as a hard copy record. Enter an alphabetic character as described previously.

12 The Instrument will print the information just entered and display: "***Printing***"Please Wait", followed by "Enter Pipette Volume:___ µL". Enter the volume (in microliters) to be dispensed and press ENTER. This volume can be any number within the range 0.1 to 5000 µL. The CLEAR key can be used to correct an entry error prior to pressing the Enter key.

13. The number of samples that can be added at this pipette volume in this vial will be displayed. The prompt: "XX Left This Vial Proceed? Yes/No", should be answered YES. It is possible to continue a calibration run in a second vial without interruption if more data points are needed (step 21).

14. The instrument will display the Range number of the Sample Solution to be used in the pipette calibration while it is taking another blank reading.

15. Locate the appropriate Sample Solution in the Reagent Kit. Mix the contents by gently inverting the bottle several times before removing the cap.

16. Using a disposable Transfer Pipette, transfer an aliquot of Sample Solution into a Sample Aliquot Container. Recap the bottle as soon as possible to avoid evaporation. Place the aliquot container in the Aliquot Container Holder to avoid any possibility of spillage. Always replace the cap lightly on the Aliquot Container to avoid evaporation. Transfer only enough Sample Solution for use in half-hour increments.

17. When the display reads "Range 1 (2, 3, 4, 5 or 6) Pipette Sample #1", aspirate a sample of Sample Solution from the Sample Aliquot Container with the pipette to be calibrated using standard pipetting technique. Open the Vial Holder Cover and dispense the Sample Solution against the inside wall of the Blank Vial slightly above the liquid level. Carefully withdraw the pipette and close the Vial Holder Cover. Place the cap lightly back on the Sample Aliquot Container between samplings to avoid evaporation.

18. When the instrument is finished reading the first sample it will display the result for that sample and prompt: "Result #1 XX.X, Pipette Sample #2". Continue pipetting samples until the desired number of data points have been collected.

19. Press the END OF RUN key. The group statistics will be computed and the results printed.

20. When using adjustable volume pipettes at multiple volumes, after the last data point for the first volume has been taken, press ENTER instead of END OF RUN to continue on to the next volume.

21. When the vial's maximum capacity is reached and additional data points for the current pipette are desired, answer YES to the prompt: "Use New Vial? Y/N/End of Vial". The instrument will prompt the removal of the current vial and the insertion of a new one. Once this is done and the Vial Holder Cover is closed, the Instrument will automatically take blank readings and prompt for the next sample to be pipetted. By inserting vials as needed, up to 50 data points for a pipette can be generated at a single volume. All of the data points will be available for use in the statistical summary.

PIPETTE CALIBRATION:

Pipette Calibration Check: continued

22. If, at the end of a run, it is determined that a data point can be validly deleted from the statistical summary, then at the prompt "Reprint Results? Yes/No", answer YES. Follow the prompts to delete the desired data point(s). Any number of data points may be deleted. The deleted data points will still appear on the printout, but an asterisk will appear beside them, indicating that they are not included in the statistical summary. An explanation for why a particular point is deleted must be attached to the printout for documentation purposes.

23. The number of data points that can be collected with each Blank Vial will depend on the volume of sample solution added, and range from 1 to a maximum of 40 for instruments using firmware version 7A2.200 or newer, and a maximum of 22 for instruments using firmware version 7A2.103 or older. Each time a pipette calibration is completed, the Instrument will display the remaining number of samples of the selected volume that can be dispensed into the vial. Within a vial's allowed capacity, additional calibration runs may be performed. The options include performing calibrations: a) at the same volume, b) at different volumes, but within the same range of Sample Solution and c) at different volumes and with a different range of Sample Solution. The prompt following completion and printing of the first calibration run will read "Another Cal w/Vial? (Yes/No)". Respond by pressing YES to continue with the next calibration run as described above.

PROCEDURE NOTES:

1. Make sure the system is kept away from anything hot or cold. The pipettes, the PCS, and all reagents should be at a stable, uniform temperature.

2. Ensure that the Reagent Kit and Instrument Calibrator Kit are at the same ambient temperature as the Instrument.

3. Keep all vials smudge free.

4. Once the Blank Reagent Vial has been inserted into the PCS it must not be turned, moved or removed until the entire pipette calibration procedure has been completed, or the vial has been consumed.

5. Once Sample Solution has been added to a Blank Vial, the Blank Vial cannot be removed and reused at a later time.

6. Use the correct Sample Solution for the specific pipette volume being tested.

7. Be sure to close the Sample Solution bottle after transferring an aliquot into a Sample Aliquot Container.

8. Always take a fresh aliquot of Sample Solution if the solution has been in the Sample Aliquot Container for over one-half hour.

9. Always place the cap (lightly) on the Aliquot Containers between pipette samplings to minimize reagent evaporation.

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

11. Blank Reagent Vials should be moved immediately from the Vial Block into the Vial Holder, giving them no chance to warm or cool along the way.

LIMITATIONS OF THE PROCEDURE:

The accuracy and precision of any pipette are affected by many factors. The condition in which a pipette is maintained, technique used in dispensing liquid, and type of pipette tips used are a few possible sources for discrepancy in results. For meaningful results, it is recommended that standard laboratory pipetting techniques are used and the specific pipette manufacturer's instructions are followed. Each department performing pipette calibration checks with the PCS should be responsible for determining the acceptable tolerance limits for inaccuracy and imprecision with their pipettes. A calibration check should be repeated once for any failed pipette to rule out operator error.

REFERENCES:

1. Exhibit A: PCS Instrument & Accessories Illustration, PCS Procedure Guide, ARTEL Document # 15A2135.
2. Clinical and Laboratory Standards Institute: Laboratory Documents: Development and Control; Approved Guideline. CLSI document #GP2-A5, Fifth Edition, Volume 26.
3. PCS2 Procedure Guide, ARTEL Document # 15A2135, or PCS3 Procedure Guide, ARTEL Document # 15A3356.
4. Curtis RH. Performance verification of manual action pipettes: part 1 & 2. Am Clin Lab 1994.

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Exhibit A: PCS® Instrument & Accessories Illustration