Standard Operating Procedure for the PCS® Pipette Calibration System Using the Artel Pipette Tracker Software

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 Using the Artel Pipette Tracker Software**

Prepared by	Effective date	Supersedes Procedure #

Review Date	Revision Date	Signature

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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.

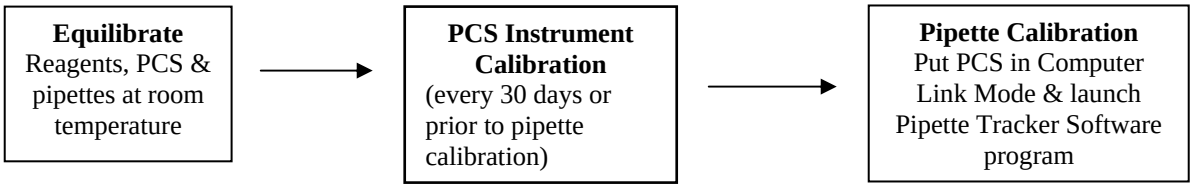
A PCS user can perform a ten-sample pipette calibration in less than three minutes. After each sampling, the Artel Pipette Tracker Software program will calculate and display the volume of liquid delivered. A printout can be generated displaying the volume of each sampled, group statistics and Pass/Fail status based on criteria that is programmed into the software by the user. To assist you in effective record-keeping and calibration scheduling, the pipette Serial number, data generated, operator, time and date, etc. are stored electronically for easy access. 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 and printer (Exhibit A Schematic)
2. PC with Pipette Tracker software installed
3. Printer for PC
4. Pipettes to be calibrated
5. Appropriate pipette tips

Materials:

1. 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)
2. PCS® Instrument Calibrator Kit containing the following:
   - CAL A vial
   - CAL B vial
   - CAL C vial
   - CAL D vial
   - Kit Insert

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 - 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 - 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 contents of the vials should be mixed by gentle inversion and 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 - 28°C), the following prompt will be displayed", "Temp out of Range; Continue (Y/N)". If NO is pressed, you will be returned to the Main Menu. If YES is pressed, you 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. If the PCS is not already connected to the PC, with the power off, connect the serial cable to the COM 1 port on the back of the PC.

2. Turn on the PCS Instrument and the PC. Upon power-on, the PCS will perform a quick system test. On the PC, launch the Pipette Tracker by clicking on the icon.

3. When the PCS system test is complete, press the Menu Scroll button until the prompt “Link to Computer” appears. Press the Yes key. The display will now read “Computer Link ready for command”.

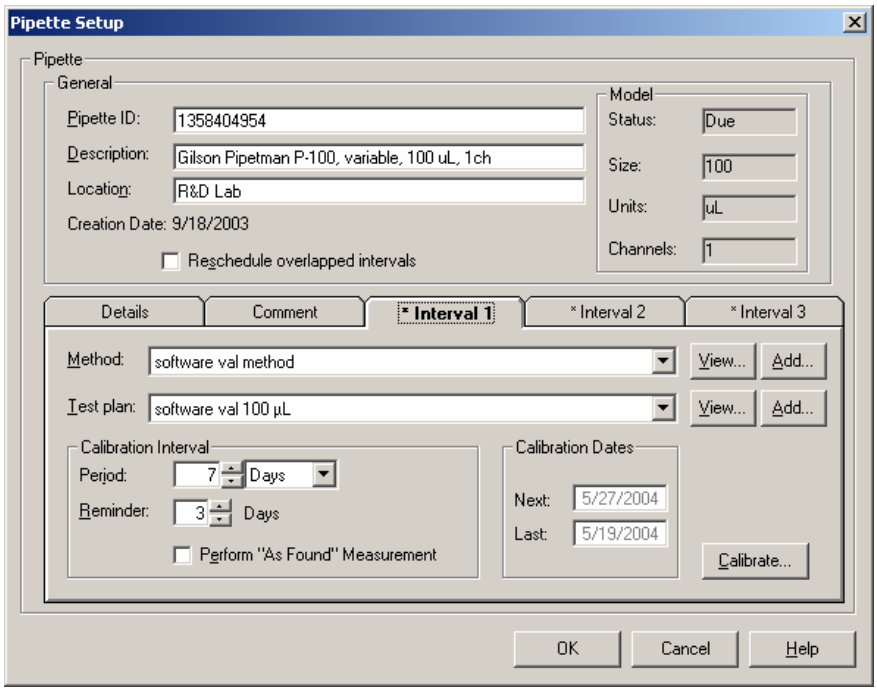
4. Before calibrating a pipette, ensure that the Reagent Kit is at the same temperature as the Instrument.

PIPETTE CALIBRATION: continued

Pipette Calibration Check:

1. On the PC, select the pipette to be calibrated from the inventory or worklist. Choose "Calibrate Selected Pipette" from the menu. If the pipette is not in the inventory, choose Setup| Pipette and then Add the pipette definition to the Pipette List before calibrating it. Be sure to complete at least one interval tab on the pipette setup dialog. (See Figure 1)

   Figure 1



2. The software will query the PCS for the last instrument calibration date and the temperature. If it has been more than 30 days since an Instrument Calibration has been performed, the program will indicate this.

3. On the Calibration dialog, select the correct Reagent Lot Code. (See Figure 2) The Lot Code is shown on the reagent vials, the reagent kit insert and on the exterior of the reagent box. The PCS maintains a library of up to 6 previously entered Lot Codes. If the Lot Code to be used matches one that was previously entered, choose the Lot Code and select Start Run and proceed to step 6. If the Lot Code is new , choose "Not Listed". Select Start Run. The program will now ask for the Lot Code information to be entered directly into the PCS as described below.

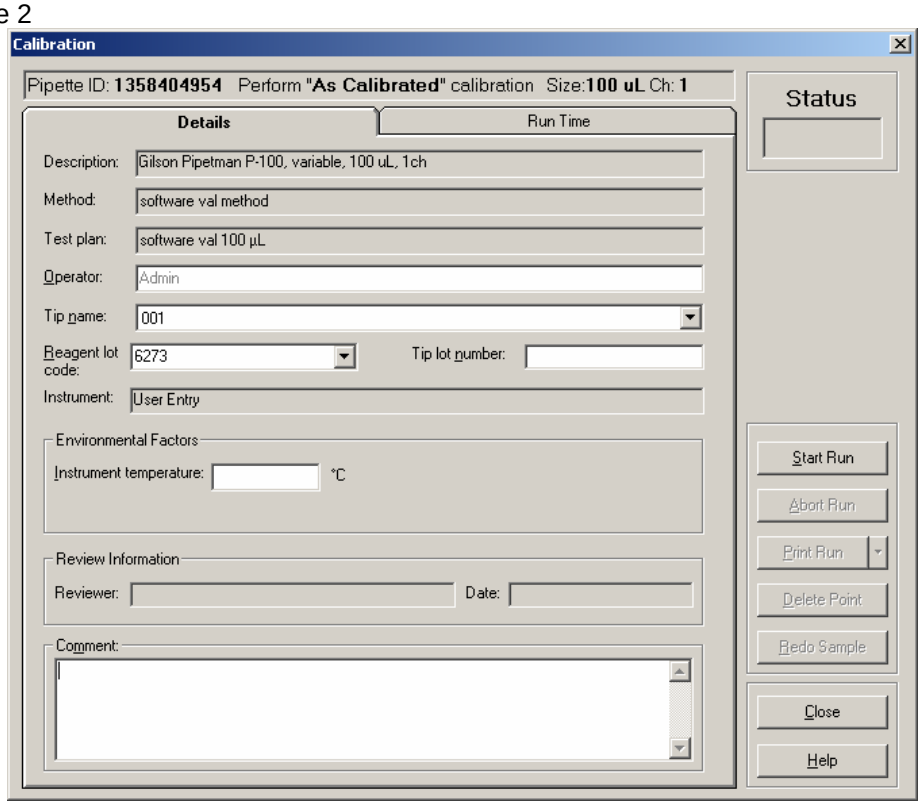
4. The Instrument will display "Enter Reagent Lot Code:__". Enter the Lot Code. The display will read "Rgt Lot Code XXXXX New Lot (Y/N)?". Press YES. Enter the Lot Code and Group Numbers found on the correct side of the Lot Code Card for your instrument. Press Enter after entry of each group of digits. At the next prompt, enter the month and year of the expiration date of the Reagent Kit as a four digit number (mmyy).

PIPETTE CALIBRATION:

Pipette Calibration Check: continued

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

Figure 2



6. The PCS 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. The instrument will take a Blank reading of the vial.

PIPETTE CALIBRATION:

Pipette Calibration Check: continued

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

11. 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.

12. 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.

13. When the instrument is finished reading the first sample, the PC will display the result for that sample and the instrument will prompt to pipette Sample #2. Data points may be redone immediately following a problem with pipetting by clicking on the REDO button after the data point is displayed. The redone data points are removed from the overall calculation; they will remain on the report, marked as voided. Continue pipetting samples until the desired number of data points have been collected.

14. After collecting the required number of data points for the pipette, the PC will display a PASS/FAIL status on the screen. Select CLOSE to save the information and to print a report.

15. When using adjustable volume pipettes at multiple volumes, after the last data point for the first volume has been taken, the software will prompt the PCS to continue on to the next volume.

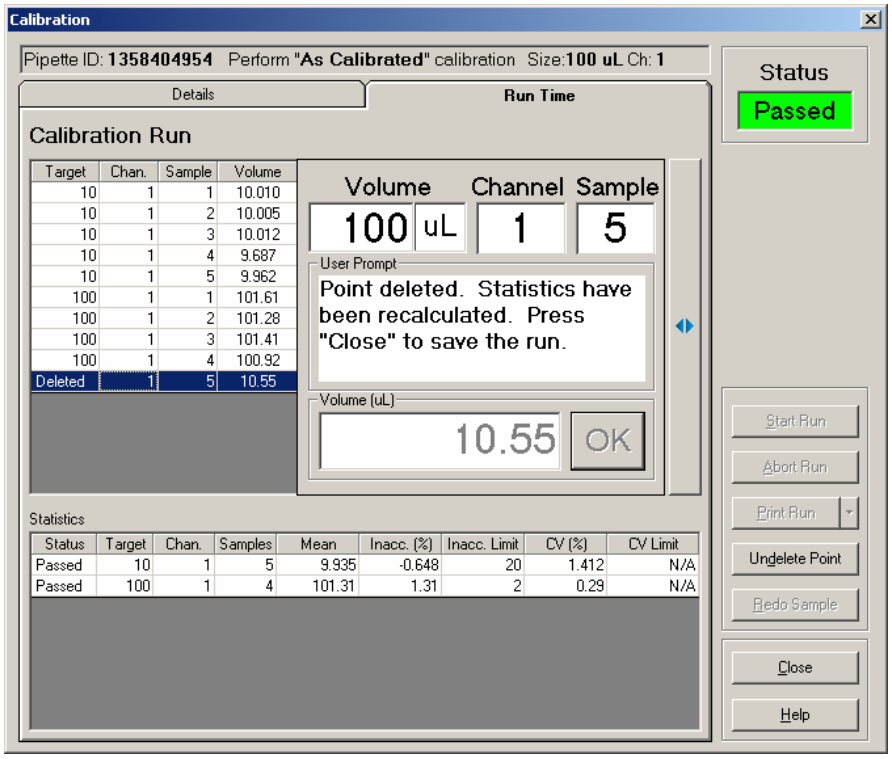
16. When the vial's maximum capacity is reached, and additional data points for the current pipette are desired, the instrument will prompt the removal of the current vial and the insertion of a new one. Once this is done and the Vial Holder Cover is closed, the Instrument will automatically take blank 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.

17. If there is a valid reason to delete a data point from the statistical summary, choose the Delete Point button at the end of the run. Select the point for deletion and fill in a reason for the deletion. (See Figure 3) The deleted data point(s) will still appear on the printout, but it will be marked as deleted and is not included in the statistical summary. To re-include a data point in the statistics, select the data point and click the Undelete Point button. The statistics will be recalculated to include the sample. You will be prompted to enter a reason each time a data point is deleted/undeleted.

PIPETTE CALIBRATION:

Pipette Calibration Check: continued

Figure 3



18. Once testing is complete, the Calibration Report should be reviewed by a supervisor. Once approved, attach a calibration sticker to the pipette with the appropriate expiration date.

19. The Pipette Tracker Software will automatically flag when the next calibration is due if the user programs the calibration period under the Setup|Pipette interval tab.

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 you are 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®2 Instrument & Accessories Illustration, PCS®2 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. PCS®2 Procedure Guide, ARTEL Document # 15A2135, or PCS®3 Procedure Guide, ARTEL Document # 15A3356.
4. Pipette Tracker User Manual, ARTEL Document # 15A4124.
5. 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