Calibration and Use of Mechanical Pipettes using the PCS® Pipette Calibration System

1. SCOPE AND APPLICATION

1.1 In order to maintain the accuracy and precision of analytical procedures, proper pipetting techniques should be followed and pipettes should be calibrated using a 3 data point or 10 data point method. This calibration procedure is based on the Artel® PCS®3 Procedure Guide and is intended for use with 0.5-1,000 µL mechanical pipettes.

2. SUMMARY OF METHOD

2.1 Proper pipetting techniques are described. Pipettes are calibrated by inserting a Blank Vial into the PCS® instrument and pipetting a predetermined volume of the appropriate sample solution into the vial. The PCS® instrument vortex-mixes the vial and determines the volume of sample solution dispensed using a colorimetric method with a high performance photometer. The volumes pipetted are compared to the expected volume to calculate accuracy and precision. Accuracy and precision are compared to Artel® and manufacturer specifications by the Pipette Tracker™ program. The PCS® instrument calibration should be checked every thirty days or prior to the next use, whichever is later.

2.2 Interferences

2.2.1 Temperature change can affect pipette volumes and PCS instrument readings. Allow pipettes to equilibrate to ambient temperature prior to pipetting. The PCS instrument will check its own temperature before a pipette calibration run is started. If the instrument temperature is outside the specified range for proper operation (18ºC-28ºC), the following prompt will be displayed: “Temp. out of range; Continue? (Y/N).” Press the **NO** key on the instrument and wait until the instrument is within the necessary temperature range before proceeding with calibration. The Bulk Reagent Kit and Instrument Calibrator Kit should be kept in the same room as the instrument to maintain temperature equilibrium. Additionally, vials should be loaded into the vial slots on the instrument at least 15 minutes prior to their use to allow them to equilibrate to the instrument temperature.

2.2.2 Fingerprints and smudges on vials can interfere with PCS instrument readings. Fingerprints and smudges that fall within the optical path will introduce errors and cause inaccurate readings. All PCS vials are cleaned and inspected prior to shipment, however, the lower portion of the vials should be cleaned with a lint free wipe, such as a Kim Wipe, prior to use. If fingerprints and smudges remain, immerse the lower portion of the vial (about 2.5 cm) into clean isopropyl alcohol, holding the vial by the cap. Remove the vial from the alcohol, and while wearing gloves, gently wipe the lower portion of the vial by rotating the vial

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against a lint free wipe. Always wear gloves when handling vials to prevent adding fingerprints and smudges.

3. EQUIPMENT AND SUPPLIES

3.1 PCS® Pipette Calibration System instrument (S/N 7-9244)

3.2 Brother P-touch 9700PC thermal barcode label printer (S/N U2345-L3Z874852)

3.3 Honeywell barcode scanner (S/N 14164B2FA6)

3.4 Mechanical pipettes for calibration – See Table 13.1.

3.5 Pipette tips of appropriate size for the pipette to be calibrated

3.6 Transfer pipettes

3.7 Sample aliquot containers

3.8 Sample aliquot container holders

4. REAGENTS AND STANDARDS

4.1 Bulk Reagent Kit (Store at room temperature (18ºC-28ºC). Keep box closed to minimize light exposure.)

4.1.1 Blank reagent (in prefilled Blank Vial)

4.1.2 Range 1 sample solution (for pipette volumes 200 to 5000 µL)

4.1.3 Range 2 sample solution (for pipette volumes 50 to 199 µL)

4.1.4 Range 3 sample solution (for pipette volumes 10 to 49 µL)

4.1.5 Range 4 sample solution (for pipette volumes 2 to 9 µL)

4.1.6 Range 5 sample solution (for pipette volumes .5 to 1.9 µL)

4.1.7 Range 6 sample solution (for pipette volumes 0.1 to 0.49 µL)

4.1.8 CAL A Calibration standard

4.2 Instrument Calibrator Kit (Store at room temperature (18ºC-28ºC). Keep box closed to minimize light exposure.)

4.2.1 CAL A instrument calibration standard

4.2.2 CAL B instrument calibration standard

4.2.3 CAL C instrument calibration standard

4.2.4 CAL D instrument calibration standard

5. PROPER PIPETTING TECHNIQUES

5.1 Adjustable and Fixed Volume Pipettes

5.1.1 For adjustable volume pipettes, check the volume setting before use. When adjusting volume make sure to move from a higher value to a lower value. If

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needed, turn the volume above the needed setting first, then turn down to the required volume. When not in use, keep the pipette at the range's lowest setting. This ensures the spring is not stretched during storage and extends the life of the pipette.

5.1.2 Aspirate and fully expel the appropriate volume of liquid 3-5 times before aspirating for delivery to pre-wet the pipette tip. Failure to do so increases evaporation within the pipette tip air space, which can cause lower delivery volumes.

5.1.3 Hold the pipette vertically and press down the control button to the first stop on the pipette. Immerse the pipette into the liquid by no more than a few millimeters to prevent carryover on the outside of the tip. Slowly and evenly release the control button, and make sure to keep the pipette vertical during this process. Holding the pipette at an angle when drawing in liquid will decrease accuracy. Wait 1-3 seconds (for 1-10 mL pipettes, wait 5 seconds) before pulling the pipette tip straight up out of the liquid. If there is a drop hanging outside the tip, it can be removed by gently wiping it on the container wall.

5.1.4 Delivery volumes are temperature dependent. Make sure liquids and pipettes are stabilized at ambient temperature. Wear gloves, hold the pipette loosely, and set down between deliveries when possible to avoid transferring body heat to the pipette.

5.1.5 Be as consistent as possible with pressure and speed of pipetting to help ensure precision.

5.2 Adjustable Volume Repeater Pipettes

5.2.1 Before inserting pipette tip, slide the filling lever all the way to the bottom and hold it there. Push the tip from below into the pipette while continuing to hold the filling lever down.

5.2.2 Select the appropriate volume by turning the volume selection wheel until it locks into the desired position.

5.2.3 Immerse the pipette tip in the liquid. Slowly and uniformly push the filling lever up to the stop.

5.2.4 Before dispensing desired volume, the pipette must be primed by placing the tip on the tube wall and actuating the dispensing lever once. This step brings the system to the basic position and should be completed independent of the volume setting.

5.2.5 To dispense, place the tip against the wall of the tube and push the dispensing lever down to the stop. The user will hear a gear movement after each discharge as the dispensing lever returns to its initial position. If this noise is not heard after dispensing, there is not enough volume in the tip to dispense the appropriate volume. Repeat previous steps to refill pipette tip.

5.2.6 Once dispensing is complete, hold the tip in the original liquid vessel and push the filling lever to the bottom to dispense unused liquid. Hold the pipette over a waste container and press both ejection keys simultaneously to release the tip.

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6. CALIBRATION AND DOCUMENTATION OF PCS® PIPETTE CALIBRATION SYSTEM INSTRUMENT

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

6.2 Turn on the PCS® Pipette Calibration System instrument and computer. Open the Pipette Tracker™ program on the computer. The uppermost window labeled "Worklist" will display devices approaching their next calibration due date. The lower window labeled "Inventory" will display all of the devices in the system and their next calibration due dates. In both windows, pipettes are identified by their device ID (serial number). The Device ID for the instrument is "Artel PCS"

6.3 Check to see if Artel PCS is in the work list and is due for calibration (>30 days). This must be completed before calibrating pipettes. If PCS has been calibrated within 30 days, then continue to pipette calibration.

6.4 Load the CAL A, CAL B, CAL C, and CAL D Vials from the Instrument Calibrator Kit (check expiration) into the vial slots on the instrument at least 15 minutes prior to their use.

6.5 Upon power-on, the instrument will perform a system test. The following prompt will be displayed: "Main Menu; Pipette Calibration." Press the **Menu Scroll** button on the instrument until "Link to Computer?" prompt appears on screen. Press the **YES** button on instrument, and "Link to Computer – Ready for Command" will be displayed.

6.6 In Pipette Tracker™, highlight and select the Artel PCS instrument in the Worklist window.

6.7 Click on "Calibrate Selected" icon (purple "running man" on far left of toolbar). The Calibration window will pop up.

6.8 Wait for the instrument temperature to display in the "Environmental Factors" section of the Calibration window.

6.9 Enter the operator information using the operator's first and last name.

6.10 Enter the Instrument Calibrator Kit Lot Code. The Calibrator Lot Code is found on the label on the inside of the Instrument Calibrator Kit box lid. It will be a four or five digit number. If the lot code is not displayed in the drop-down menu, choose "not listed." The instrument will prompt for the new lot code information. Enter the information on the box lid.

6.11 Enter the Instrument Calibrator Kit serial number and expiration date, located on the exterior of the Instrument Calibrator Kit box.

6.12 Click the **Start Run** button.

6.13 The instrument will take a series of readings and then display a prompt to insert the CAL A Vial. Use the CAL A provided in the Instrument Calibrator Kit (not the Bulk Reagent Kit). Insert it into the instrument and close the door when prompted by the instrument.

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6.14 When the readings are complete, the instrument will prompt to remove CAL A, followed by a prompt to insert the CAL B Vial and close the door. CAL B, CAL C and CAL D are sequentially inserted, following the prompts. Data will not display in the program Calibration window until the calibration is complete.

6.15 The "Status" section of the Calibration window will be green and display "Passed" if the calibration passes. When an instrument calibration is successful, the date of the last instrument calibration will change to the current day. This date is displayed on the footer of subsequent pipette calibration reports. If the instrument fails its calibration, the date will not change. If the calibration fails and the "Status" section is red, clean all the CAL vials and repeat the calibration.

6.16 When calibration is complete, click the **End of Run** button.

6.17 Select the **Print Run** button.

6.17 When report is displayed in print preview, select File/Export from the menu. Click the **...** button on the following page and choose the correct file path to save the .pdf file. Refer to Section 8 to save the file in the appropriate folder and use the correct naming convention. The device ID is PCS9244.

7. CALIBRATION AND DOCUMENTATION OF MECHANICAL PIPETTES

7.1 General Information

7.1.1 The worklist in Pipette Tracker™ should be checked on the first of each month to determine which pipettes' calibration due dates are approaching.

7.1.2 CAL A (from the Bulk Reagent Kit) and Blank Vials should be loaded into the vial slots on the instrument at least 15 minutes prior to their use. Four vials at a time can be loaded into the vial slots.

7.1.3 Before calibrating pipettes, ensure that the PCS® instrument has been calibrated within the last 30 days by checking the worklist/inventory. See Section 6 for instrument calibration procedure.

7.1.4 Each pipette is identified by its serial number which is clearly printed on the pipette and on the barcode ID label produced by the Artel® Pipette Tracker™ program. The serial number is identical to the Device ID used in the Artel® Pipette Tracker™ program.

7.2 Pipette Calibration Setup

7.2.1 In Pipette Tracker™, highlight and select the device to calibrate (from worklist or inventory list). Alternatively, the device may be selected by scanning the barcode ID label with the barcode scanner.

7.2.2 Click on the "Calibrate Selected" icon (purple "running man" on far left of toolbar). The Calibration window will pop up.

7.2.3 Select "Artel PCS" as the Standard Name in the "Measurement Standards" section. The PCS serial number (9244) will appear beside the selection. Once connected, the instrument temperature will also be displayed in the "Environmental Factors" section.

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7.2.4 Select the Reagent Lot Code from the drop down menu. The Lot Code is shown on the Reagent Vials, 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.

7.2.4.1 If the Reagent Lot Code is not displayed, choose "Not Listed" in the drop down menu. Once the calibration run is started, the instrument display will prompt: "Rgt Lot Code XXXXX; New Lot? (Y/N)." Press "YES" if entering a new Lot Code. The Group Numbers found on the correct side of the Lot Code Card for the instrument type will need to be entered on the 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 indicated on the Lot Code Card in the format mmyy (e.g. 0811 for August 2011). The following prompt will be displayed: "Lot Code: XXXXX; Correct? (Y/N?)." Press "YES" to confirm.

7.2.5 Enter operator information using the operator's first and last name in the "Details" tab.

7.2.6 Click the **Start Run** button to begin the calibration. The instrument display will give step by step directions throughout the calibration process.

7.2.7 The instrument will perform a brief self-calibration. The following prompt will be displayed: "Self-Calibration; Please Wait," followed by "Insert CAL A Vial."

7.2.8 Remove the CAL A Calibration Standard from the vial slot on the instrument. Open the Vial Holder Cover on the instrument and insert CAL A into the Vial Holder, pressing it down until it meets the Vial Holder. Never remove the cap of the CAL A Calibration Standard. When prompted by the instrument, close the Vial Holder Cover.

7.2.8.1 Use the CAL A from the Reagent Kit in use, not the CAL A from the Instrument Calibrator Kit. The label for this vial should face toward the front of the instrument, with the vertical line on the vial facing forward.

7.2.9 The instrument will perform a Zero Check while displaying: "PCS Zero Check; Please Wait." When prompted, remove CAL A and place it into the Reagent Kit for future use.

7.2.10 The following prompt will be displayed on the instrument: "Remove Cap and Insert Blank." Remove a Blank Reagent Vial from the vial slot, remove the cap, and insert into the Vial Holder until it contacts the bottom of the Vial Holder.

7.2.10.1 Each vial contains a predetermined amount of liquid and spillage will reduce accuracy.

7.2.10.2 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.

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7.2.11 When prompted, close the Vial Holder Cover. The instrument will read the Blank while displaying: “Reading Blank; Please Wait.” When finished, the instrument will display “Pipette Sample #1.”

7.3 Pipette Calibration

7.3.1 Locate the appropriate Sample Solution in the Reagent Kit. The Sample Solution Range should correspond to volume being pipetted and will be indicated by the instrument and the Calibration window in the program. Mix the contents by inverting the bottle several times before removing the cap.

7.3.2 Using a disposable Transfer Pipette, transfer an aliquot of Sample Solution into a Sample Aliquot Container. Transfer Pipettes and Sample Aliquot Containers are found in the Plastics Kit. Place the container into the Aliquot Container Holder to reduce the possibility of spillage. Place the cap lightly on the Sample Aliquot Container to avoid evaporation, and replace the cap on the Sample Solution Bottle to avoid evaporation. Transfer only enough Sample Solution for use in half-hour increments. The total volume of Sample Solution required depends on the sample volume and the total number of data points being generated. The Calibration window will show a list of the sample volumes and number of data points needed for the pipette being calibrated. The volume graduations on the Transfer Pipette as well as the 1 mL mark on the Sample Aliquot Container can be used as a guide.

7.3.2.1 When handling open Blank Reagent Vials and open Sample Solutions, be very careful not to spill any liquid on or along the edges of the instrument’s keypad.

7.3.3 Aspirate the Sample Solution from the Sample Aliquot Container with the pipette to be calibrated, using proper pipetting techniques. Place the cap lightly back on the Sample Aliquot Container between samplings to avoid reagent evaporation.

7.3.4 Lift the Instrument Vial Holder Cover and dispense the Sample Solution into the Blank Reagent Vial.

7.2.10.1 The Sample Solution should be dispensed against the inside wall of the vial with the pipette tip placed above the meniscus but below the shoulder of the Blank Vial. The vortex mixing action will wash the solution from the inside wall of the vial.

7.3.5 Once the sample is dispensed, carefully withdraw the pipette and close the Vial Holder Cover. The instrument will display: “Mixing Sample #1; Please Wait,” followed by: “Reading Sample #1; Please Wait.”

7.3.6 After reading Sample #1, the instrument will display the results of the reading, and will prompt to dispense the next sample. Results will also be displayed in the Calibration window. Follow the same procedure for each subsequent sample addition. After each subsequent sample addition, the instrument will mix, read, and display results for each sample.

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7.3.7 Pay close attention to the "target value" displayed in the Calibration window when calibrating multiple volumes on an adjustable pipette. This value represents the volume to be pipetted for the given sample, and will change every 3 or 10 samples depending on the calibration method being used. Make sure to check the units of the target value. It may be displayed in mL or µL.

7.3.8 Pay close attention to the Sample Solution Range displayed on the instrument and in the Calibration window when calibrating multiple volumes on an adjustable pipette. Different Range solutions may be needed for different sample volumes on the same pipette.

7.3.9 When the Blank Vial's maximum capacity is reached and additional data points are needed for the current pipette, answer YES to the "Use New Vial? (Y/N)" prompt on the instrument. The instrument will prompt to remove the current vial and insert another one. It will automatically take the Blank Vial readings and prompt for the next sample addition. This process can be repeated with subsequent vials.

7.3.10 If accuracy and precision tolerance standards are met once all samples for a given volume are dispensed, the "Status" box in the Calibration window will turn green and display: "In Tolerance." Conversely, if accuracy and/or precision tolerance standards are not met once all samples for a given volume are dispensed, the "Status" box will turn red and display: "Out of Tolerance." If the accuracy and/or precision tolerance standards are out of tolerance for a given volume, follow the corrective action procedure as described in Section 9.

7.3.11 When all samples have been dispensed, click "End of Run" to complete the calibration.

7.4 Documentation

7.4.1 Once the calibration run is competed, a calibration label with a barcode and the date of the calibration will automatically print from the barcode label printer. Remove the old calibration label (not the barcode ID label) and affix the new calibration label in its place. The pipette calibration report will automatically export. Refer to Section 8 to save the file in the appropriate folder and use the correct naming convention.

7.4.2 Click the **Close** button to close the Calibration window. If the calibration was "In Tolerance", the pipette will be removed from the worklist and will only appear in the inventory list until it is due again from calibration. If calibrating another pipette follow Section 7 again, except the instrument will not prompt for Cal A again.

7.4.3 When calibrations are complete, turn off the PC, barcode label printer, and instrument. Place the cap back on the blank vial and return to the reagent kit box. Place the cover back on the instrument.

8. DATA ARCHIVAL

8.1 All calibration files should be stored in .pdf format.

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8.2 For Molecular group pipettes (see Table 13.1), save all files in the appropriate dated folder in the following directory: \\Fldep1\dear\Labs\Lab3\Biology\Artel PCS\Calibration PDF files.

8.3 For BenchBio group pipettes, save all files in the Artel PCS Pipette Calibration Export Files folder in the folder for the current year in the following directory: \\fldep1\dear\labs\lab5\Biology\Bench Common\Calibrations\Pipette.

8.4 For all PCS instrument calibration files, save in the following folder: \\Fldep1\dear\Labs\Lab3\Biology\Artel PCS\PCS Instrument Calibration.

8.5 All calibration files should have the following naming convention which includes the Device ID (pipette serial number), date, and calibration method (IC-Instrument Calibration, CC-Control Calibration, QC-Quick Check):

8.5.1 Filename: DeviceID-YYMMDD-QC.pdf

8.5.2 When saving in Pipette Tracker, always make sure to add the ".pdf" extension. If it is not added, the file will not be viewable.

9. QUALITY CONTROL AND CORRECTIVE ACTION

9.1 If a sample point needs to be re-read due to user error, highlight appropriate sample(s) in Pipette TrackerTM and select "Redo Sample." Follow the prompts on the instrument to redo the sample point. Enter the reason for redoing the sample point in the box that appears (e.g. pipette tip fell into Blank Vial, instrument door was closed prematurely, wrong sample solution was used, etc.).

9.2 If a pipette fails to pass a pipette calibration run, save the pipette calibration report as described in 7.23 and notify a supervisor. A repeat pipette calibration run may be performed if the calibration failure was due to user error.

9.3 All pipettes or dispensers that can no longer be used for analytical purposes or are broken should be removed from the lab. All uncalibrated pipettes that are in use in the lab for purposes like dispensing media must be approved for the intended use by the supervisor. They must be clearly labeled "NOT CALIBRATED" and state the approved use.

9.4 If accuracy limits fail but precision limits pass, check the difference between the average volumes measured and the volume setting for each required volume. If the difference is about the same for each weighing, then the pipette may need an adjustment. If precision fails, make sure to use proper pipetting techniques and be consistent with each weighing. Precision cannot be adjusted on the pipette. Precision is mostly affected by handling of the pipette, but can also be due to worn parts that may need replacing. For any type of failure, always check the pipette for leaks and cleanliness.

10. MAINTENANCE

10.1 Cleaning: Before use, always check the outside of the pipette for cleanliness. If needed, wipe the outside of the pipette with 10% bleach solution and then with deionized water to remove bleach residue. If the inside is contaminated (liquid pulled into the pipette),

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follow the manufacturer's instructions to disassemble the lower part of the pipette and clean with deionized water only. Remove any old grease, before lubricating the piston sealing rings.

10.2 Adjustment: Determine the approximate amount of adjustment needed. Subtract the set volume from the actual volume measured for each of the volumes checked in the calibration. Use the average of these results for the adjustment. Follow manufacturer's instructions for adjustment.

11. SAFETY/HAZARDOUS WASTE MANAGEMENT

11.1 Refer to Material Safety Data Sheet (MSDS) for proper disposal. All PCS Reagents are non-hazardous and aqueous-based.

12. REFERENCES

12.1 PCS3 Procedural Guide

12.2 Pipette Tracker User Manual QA Manual

12.3 Artel's 10 Tips to Improve your Pipetting Technique

12.4 Eppendorf Multipipette plus/Repeater plus Operating Manual

13. TABLES, DIAGRAMS

Table 13.1 Pipettes calibrated with PCS System

Group	Pipette ID	Volume (µL)	Type
BenchBio	1765876	10-100	Eppendorf Reference
BenchBio	4431431	100-1000	Eppendorf Reference
BenchBio	49197	100	Hamilton
Molecular	M20435B	0.5-10	Eppendorf Research Plus
Molecular	L14911B	2-20	Eppendorf Research Plus
Molecular	L15097B	2-20	Eppendorf Research Plus
Molecular	J1B706B	10-100	Eppendorf Research Plus
Molecular	J1B721B	10-100	Eppendorf Research Plus
Molecular	J33952D	20-200	Eppendorf Research Plus
Molecular	M24765B	20-200	Eppendorf Research Plus
Molecular	M24817B	20-200	Eppendorf Research Plus
Molecular	I32865B	100-1000	Eppendorf Research Plus
Molecular	I32903B	100-1000	Eppendorf Research Plus
Molecular	I32941B	100-1000	Eppendorf Research Plus

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Molecular	I32943B	100-1000	Eppendorf Research Plus
Molecular	I14313B	1-10000	Eppendorf Repeater Plus
Molecular	K25088C	1-10000	Eppendorf Repeater Plus
Molecular	O117790	0.5-10000	Eppendorf Xplorer

Appendix of Changes

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