Calibration and Use of Mechanical and Disposable Pipettes

1. SCOPE AND APPLICATION

1.1. To maintain the accuracy and precision of analytical procedures, proper pipette techniques should be followed, and pipettes should be calibrated quarterly.

2. SUMMARY OF METHOD

2.1. Proper pipetting techniques are described. Pipettes are calibrated using the known density of water. An aliquot of water is pipetted and weighed. The density of water is used to calculate the actual volume pipetted and this is compared to the expected volume to calculate accuracy and precision. Accuracy and precision are compared to manufacturer's limits. Calibration for disposable pipettes and mechanical pipettes may be performed using the Sartorius Cubis II balance in the qPCR laboratory. Refer to SOP BB-39 for calibration using the Sartorius system..

3. DEFINITIONS

3.1. Nominal Volume – The largest volume given by the manufacturer in an adjustable volume pipette.

4. EQUIPMENT AND SUPPLIES

4.1. Weighing vessel

4.2. Mettler Toledo S/FACT (S/N 1128150043) – Calibration and use per SOP TA-06.38.

4.3. Mettler Toledo XA105DU (S/N B314195659) – Calibration and use per SOP BB-036.

4.4. Certified digital thermometer

4.5. Pipette pump

4.6. Disposable and/or adjustable pipettes to calibrate

4.7. Pipette tips

4.8. Calibration And Use of Mechanical Pipettes Using the Sartorius Cubis® II Balance – calibration and use per SOP BB-39.

5. REAGENTS AND STANDARDS

5.1. Deionized Water (DI Water)

6. PROPER PIPETTING TECHNIQUES

6.1. For adjustable volume pipettes, check the volume setting before use. When adjusting volume, make sure to move from a higher volume to a lower volume. If needed, turn the volume above the needed setting first, then turn down to the required volume.

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6.2. Aspirate and fully expel the appropriate volume of liquid at least 3 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.

6.3. 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 5 seconds before pulling the pipette tip out of the liquid. If there is any liquid remaining on the outside of the pipette tip, remove it by gently wiping it on the container wall.

6.4. To dispense the liquid, hold the pipette tip at an angle against the wall of the vessel and press the control button slowly and evenly until the first stop. Wait until the liquid stops flowing (1-2 seconds) and then continue pressing the control button until the second stop. Pull the pipette tip slowly out of the vessel while wiping it against the vessel wall. Do not release the control button until the pipette is outside of the vessel.

7. CALIBRATION CHECK OF DISPOSABLE PIPETTES

7.1. Check the calibration of the balance and record in the daily use log for the balance. See SOP TA-06.38 and/or SOP BB-036.

7.2. Select one pipette (at random) from each new lot received to check calibration (50 mL, 10 mL, 5 mL, 2 mL and 1 mL). With the aid of a pipette pump, slowly draw room temperature DI water to the maximum volume of the pipette being calibrated (50 mL, 10 mL, 5 mL, 2 mL, and 1 mL) and dispense volume into the appropriate tared container on a certified balance. Make sure that the sliding glass sides of the balance are fully closed to get an accurate reading.

7.3. Record the results for volumes of 50 mL, 10 mL, 5 mL, 2 mL, and 1 mL into the **LIMS Laboratory Logbook**, *PIPET-FUN_CAL_LOG*, and identify whether the test was accepted or rejected. The error of calibration for a given manufacturer's lot must not exceed 2.5%. If the pipette calibration results are rejected, discard that pipette. The test shall be repeated one additional time using another pipette from the same lot. If this test fails, the department will reject the entire lot number received and contact the vendor for a new lot number.

8. CALIBRATION AND DOCUMENTATION OF MECHANICAL PIPETTES

8.1. Each pipette is identified by its serial number which is clearly printed on the pipette and documented in the Pipette Calibration worksheet. The Pipette Calibration worksheet template is located in the following folder:
\\floridadep\data\DEAR\Labs\Lab5\Biology\BenchCommon\Calibrations\Pipette

8.2. Every three months, a new copy is made and renamed by the month and kept in the current year's folder.

8.3. Let the pipettes and DI water that will be used for the calibration rest near the balance for at least 2 hours to ensure that they equilibrate to the same temperature.

8.4. Open the Pipette Calibration worksheet for the current quarter. Select the "Enter Weights" tab. Locate the pipettes to be calibrated and enter the analyst, date, and balance serial number.

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8.5. Check the calibration of the balance and record in the daily use log for the balance. See SOP TA-06.38 and/or SOP BB-036.

8.6. Measure the temperature of the DI water and enter the thermometer serial number and temperature (rounded to the nearest 0.5°C) in the worksheet.

8.7. Place the weighing vessel (use a glass beaker of the appropriate size) on the balance plate and tare the balance. With a new pipette tip, aspirate the DI water at the maximum volume and empty it back into the DI water reservoir. Repeat 5 times to create a moisture balance inside the pipette. Replace the pipette tip and aspirate three times to pre-wet the new tip.

8.8. Starting with the minimum required volume, pipette DI water 10 times into the weighing vessel and record weight after each rep in the worksheet. Allow the balance to stabilize before recording each weight. Make sure that the sliding glass sides of the balance are fully closed after each repetition to get an accurate reading. Do not re-zero the balance after every rep. Enter the cumulative weight. Tare the balance only when starting a new volume. Do this for each required volume. The required volumes are documented in the worksheet. Most pipettes require three volumes (nominal volume and 50% and 10% of nominal volume). Some pipettes require only two volumes (maximum and minimum volumes). This depends on the manufacturer instructions and limits. If only one value is calibrated, the use of the pipette is restricted to this volume.

8.9. When all the weights for the pipette are complete, go to the "Pipette Calibration" tab and check the accuracy and precision results. This page will automatically calculate accuracy and precision limits for each pipette. If the calibration is acceptable it will display "PASS" in the Pass/Fail column of the spreadsheet. If accuracy or precision exceed the manufacturer's specifications, it will display "FAIL". If all the calibrations are acceptable, save the calibration spreadsheet. If a calibration is not acceptable, follow the corrective action procedure as described in Section 9.

8.10. After the calibration is completed, a LIMS generated label must be printed and attached to the pipette. Open the LIMS program. Go to **Laboratory\Receiving\Print Preparation Labels**. Under the "Options" menu, select "Four Lines of Text (Left)". Enter the text of the first line of the label in the box at the bottom of the "Print Prep Labels" window. Each time the "Enter" key is pressed a small new window appears where you can enter the next line of text. The label must contain the pipette ID, calibration date, the analysis group the pipette is assigned to, and the initials of the analyst. Additionally, if a variable pipette was only checked at one volume, add the volume checked. Example labels are shown below:

PIP ID EH75658
CAL DATE 11/10/17
250 µL only
BOD DT

PIP ID J18706B
CAL DATE 12/20/17
PCR
DT

9. QUALITY CONTROL AND CORRECTIVE ACTION

9.1. Do not over-write calibration results. If a calibration result is not acceptable, the "PASS/FAIL" column of the calibration spreadsheet shows "FAIL" in red letters. In this case, the calibration must be repeated. Go to the "Repeat Weights" tab of the worksheet. The left frame of

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this sheet is clearly marked in red. Perform a new calibration as described in Section 8. Check the calibration results in the "Repeat Calibration" tab. Add a comment in the "Comments" column of the worksheet describing any maintenance that may have been performed on the pipette. If the calibration passes, save the file, and print a calibration label for the pipette as described in Section 8.

9.2. If the calibration fails again, notify your supervisor, and perform further maintenance or re-adjustments. Go to the "Corrective Weights" tab of the calibration worksheet. The left frame of this sheet is clearly marked in yellow. Use this tab to calibrate the pipette for the third time following the procedure outlined in Section 8. After the calibration is completed, a comment must be entered in the "Comments" column of the spreadsheet detailing the corrective action performed. If a pipette could not be calibrated successfully, it must be taken out of service.

9.3. 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 similar 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.

9.4. 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.

9.5. Do not use any other template for performing the quarterly pipette calibrations. The quarterly pipette calibrations are due by the 20th of the month in which they are due (March, June, September, and December). A supervisor will check the calibration worksheet for correctness and completeness. This allows for enough time until the end of the quarter to correct any problems that may be observed at that time.

9.6. Hand-written pipette labels are not acceptable. Please refer to Section 8.9 for details.

9.7. After the calibration worksheet is approved by the supervisor, it is converted to pdf format for archival on the Biology drive at \\floridadep\data\DEAR\Labs\Lab5\Biology\BenchCommon\Calibrations\Pipette in the appropriate yearly folder.

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 DI water to remove bleach residue. If the inside is contaminated (liquid pulled into the pipette), follow the manufacturer's instructions to disassemble the lower part of the pipette and clean with DI water only. Remove any old grease before re-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.

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11. SAFETY/HAZARDOUS WASTE MANAGEMENT

11.1. Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management.

11.2. Read MSDS information for reagent handling and disposal.

11.3. Glass pipettes should be placed in designated glass boxes for disposal.

12. REFERENCES

12.1. Standard Methods 9020-2017

12.2. NELAC Quality Systems Manual

12.3. Eppendorf Research Plus Operating Manual

12.4. Eppendorf Standard Operating Procedure for Pipettes

12.5. FDEP Laboratory Quality Manual https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

12.6. FDEP Laboratory Health and Safety Plan https://floridadep.sharepoint.com/dear/Shared%20Documents/FDEP%20Health%20%20Safety%20Plan%20B%20-%20June%202021.pdf

12.7. SOP BB-036-1.6 *Calibration, Use, and Maintenance of Mettler Toledo XA105DU Balance*

12.8. SOP TA-06.38-1.7 *Calibration, Use and Maintenance of Mettler Toledo PB403-S/FACT Balance*

12.9. SOP BB-39 *Calibration and Use of Mechanical Pipettes Using the Sartorius Cubis II Balance*

APPENDIX OF CHANGES

02/18/2013 Changed SOP to new format and included calibration of mechanical pipettes.

01/23/2015 Formatted for 508 compliance

03/12/2015 Referenced the Artel Pipette Calibration System and corresponding SOP; updated file directories; monthly calibration checks have been changed to quarterly checks

03/14/2017 Added 2 mL and 50 mL disposable pipettes, removed references to pipettes less than 1 mL, updated hyperlinks.

03/15/2019 Updated balance SOP reference.

03/25/2020 Reformatted, added small details to add clarity and updated SOPs for calibration, use and maintenance of balances.

03/18/2021 Updated SOP references and hyperlinks.

02/17/2021 Updated SOP references and hyperlinks.

01/03/2023 Updated link for FDEP Laboratory Health and Safety Plan

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12/19/2023	Updated balance SOP references, Standard Method reference, and hyperlinks. Edited section 9.2 to add clarity to the steps that need to be followed on that portion of the SOP.
01/29/2026	SOP was updated to align with current equipment usage by incorporating information related to the Sartorius Cubis II balance.

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