CALIBRATION AND VERIFICATION OF MECHANICAL AND DISPOSABLE PIPETTES IN THE BIOASSAY–AGP LAB

1. SCOPE AND APPLICATION

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

2. SUMMARY OF THE METHOD

2.1. 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.

3. EQUIPMENT AND SUPPLIES

3.1. Weighing vessel (flask, beaker, tube, weigh-boat, etc.)

3.2. Analytical balance: Mettler Toledo 403 TS/00 (SN B815673069)- Calibrate and use per SOP TA-06.34 (accurate to 0.001 g)

3.4. Certified Digital thermometer (Calibrate per SOP TA-06.13.)

3.6. Computer with the *Pipette calibration* Excel spreadsheet

3.7. Pipettes to be calibrated/verified

4. REAGENTS AND STANDARDS

4.1. Deionized (DI) water

5. PROCEDURE AND DOCUMENTATION

5.1. All pipettes are assigned unique ID numbers. These ID numbers are documented on the pipette labels and in the appropriate pipette calibration spreadsheet. If a new or different pipette is put into use, notify supervisor to modify the spreadsheet accordingly.

5.2. The calibration spreadsheet template for the Bioassay-AGP workgroup is located in the Pipette Calibration folder in \\floridadep\data\DEAR\Labs\Lab5\Biology\BenchCommon\ToxCommon\QC\Pipette Calibration\Quarterly Pipette Calibration. Open the spreadsheet and save-as the current month/year. On the bottom left side of the file there are two tabs

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titled “Pipette Calibration” and “Calc”. Make sure the Pipette Calibration tab is active. Scroll to the top of the sheet and read the instructions carefully.

5.3. In the calibration spreadsheet, locate the pipette or set of pipettes that you are going to calibrate. For each pipette the volumes to be used for the calibration are already entered. Always calibrate all volumes given in the spreadsheet. If any changes to the spreadsheet are needed, contact the supervisor. Record the analyst, date, balance serial number, thermometer ID and temperature of the DI water used for the calibration. The DI water should be in a flask with the thermometer.

Note: When using variable volume pipettes the accuracy at the maximum, middle and minimum values are checked. If only one volume is calibrated the use of the pipette is restricted to this volume. The volume must be documented on the pipette label (see 5.7). Refer to Section 6 of SOP MB-03.3 for proper pipetting techniques. Refer to Section 7 of SOP MB-03.3 for calibration of disposable pipettes.

5.4. Check the calibration of the balance to be used (see SOP TA-06.34). Bracket check weights around expected values.

5.5. Locate a weighing boat/vessel that can hold the volume of DI water to be pipetted. Weigh the weighing vessel and tare balance. If you are calibrating a variable volume pipette, make sure the pipette is set to the correct volume. Pipette five aliquots of DI water into the vessel and weigh the contents. Record the final weight in the column of the spreadsheet called “Weight of 5 Reps (g)”.

5.6. Check the “% Difference” and “Pass/Fail” columns of the spreadsheet. The percent difference must not be greater than the manufacturer's specifications for the pipette. If the calibration is acceptable “PASS” will automatically appear in the “Pass/Fail” column. If the calibration is not acceptable “**FAIL**” will automatically appear in red letters in the “Pass/Fail” column. If all the calibrations are acceptable, save the calibration spreadsheet and notify your supervisor immediately to protect the worksheet. If a calibration is not acceptable follow the corrective action procedure as described in Section 6 of this SOP.

5.7. After the calibration is completed, label the pipette with the calibration information using either laboratory tape or a LIMS generated label. To generate a LIMS label: Open the LIMS program. Go to the “Laboratory” menu and under “Technical” select the “Prep Worksheet Manager”. There are six icons to select from. Choose the third one from the left, depicting a small label, with the name “Print Prep Labels”. After selecting it, a new window will open. In this new window, under the “Options” menu, select “Four Lines of Text”. Enter the text of

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the first line of the label in the box at the bottom of the "Print Prep Labels" window. Each time the "Enter" key is clicked a small new window appears where you can enter the next three lines of text. The label must contain the pipette ID, calibration date, the lab (TOX-AGP) 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. In lieu of using LIMS to label, the same information can be written on a piece of tape. Example labels are shown below:

PIP ID 1234
CAL DATE 09/25/15
1000 uL only
AGP

PIP ID 3992086
CAL DATE 12/20/11
C. dubia Feeding
TOX

6. QUALITY CONTROL AND CORRECTIVE ACTION

6.1. Do not over-write calibration results. If a calibration result is not acceptable the "PASS/FAIL" column of the calibration spreadsheet will show "**FAIL**" in red letters. In this case, the calibration must be repeated. After 5 repeats failures, activate the "Corrective Action" tab of the worksheet. Perform a new calibration as described in Section 5 of this SOP. If the calibration passes at this point, add a comment in the "Comments" column of the worksheet describing any maintenance that may have been performed on the pipette.

6.2. In case the calibration fails again, perform further maintenance or re-adjustments on the pipette. After the calibration is completed, a comment must be entered in the "Comments" column of the spreadsheet detailing the corrective action performed. Notify supervisor if a pipette could not be calibrated successfully. It must be taken out of service.

6.3. All pipettes or dispensers that can no longer be used for analytical purposes or are broken should be removed from the lab. All non-calibrated pipettes that are in use in the lab for purposes such as transferring concentrated acids or soaps/surfactants must be approved for the intended use by the supervisor. They must be clearly labeled "NOT CALIBRATED" and state the approved use.

6.4. Do not use any other template for performing the Quarterly pipette calibrations. The Quarterly pipette calibrations are due by the 20th of the first month of each three-month period. Each pipette should have a permanent label with this calibration due date information. A supervisor will check the calibration worksheet for correctness and completeness. This allows for enough time until the end of the calibration month of the quarter to correct any problems that

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may be observed at that time.

6.5. After the calibration worksheet is approved by the supervisor, it should be converted to pdf format for archiving at \\floridadep\data\DEAR\Labs\Lab5\Biology\BenchCommon\ToxCommon\QC\Pipette Calibration\Quarterly Pipette Calibration.

7. CALCULATION

The following equation is used to calculate the percent difference between the nominal volume pipetted and the calculated volume. The spreadsheet will calculate this value automatically. The result is recorded in the "% Difference" column.

$$\%D = |(V_e - V_a)/V_e| * 100$$

W_a = Average weight of water = W_n/n

V_a = Average volume = W_a/Density of water at given temperature (mL)

V_e - Volume of water according to pipette

n - Number of aliquots pipetted

W_n - Weight of n aliquots of water

%D - Percent difference between volume of water according to the pipette and the calculated volume

The Density of water is used as reported in: CRC Handbook of Chemistry and Physics, 75th ed., Ed. David R. Lide, CRC Press Inc., 1994

Example: 20 ºC: 0.9982 g/mL

8. REFERENCES

8.1. CRC Handbook of Chemistry and Physics, 75th ed., Ed. David R. Lide, CRC Press Inc., 1994.

8.2. DEP SOP CM-011, *Gravimetric Analysis: Analytical Weight and Balance Calibration.*

8.3. DEP SOP TA-06.34, *Calibration and Use of Mettler Toledo 403 TS/00 Balance.*

8.4. DEP SOP TA-06.13, *Thermometer Accuracy Check and Use.*

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8.5. DEP SOP BB-36, *Calibration, Use, and Maintenance of Mettler Toledo XA105DU Balance.*

8.6 DEP SOP MB-03.3, *Calibration and Use of Mechanical and Disposable Pipettes.*

Appendix of Significant Changes

08/30/2018	Corrected balance information. Changed font to Calibri.
09/13/2018	Deleted MS from Balance name. (M. Faircloth)
08/28/2019	Updated links
08/31/2020	Updated equipment section. Updated titles for equipment and reagents.
07/07/2021	Checked for grammar issues. Updated SOP information and removed a reference that no longer exists.
08/12/2022	Checked for grammar and removed equipment that is no longer used.
08/11/2023	Updated links. Updated QC and Corrective Action section to reflect current excel sheet. Added mentioned SOPs.

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