CALIBRATION OF AUTO-PIPETTES AND DISPENSERS

1. SCOPE AND APPLICATION

In order to maintain the accuracy of analytical procedures, pipettes should be calibrated at least once a month.

2. SUMMARY OF THE METHOD

Pipettes and dispensers 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. APPARATUS AND EQUIPMENT

3.1. Weighing vessel

3.2. Deionized (DI) water

3.3. Analytical balance (accurate to 0.0001 g)

3.4. Calibrated thermometer

4. PROCEDURE AND DOCUMENTATION

4.1. All pipettes are assigned unique ID numbers. These ID numbers are documented on the pipette labels and in the appropriate pipette calibration spreadsheet. If you want to start using a new or different pipette, notify your supervisor to modify the spreadsheet accordingly.

4.2. There is one calibration spreadsheet template for the Nutrients workgroup and one for the General Chemistry workgroup located in the current year\current month subfolder in the Pipette Calibration folder on the Nutrients drive at \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\. Open the spreadsheet for the current month. On the bottom left side of the file there are three tabs titled “Pipette Calibration”, “Repeat Calibration”, and “Corrective Action” respectively. Make sure the Pipette Calibration tab is active. The spreadsheet is organized by chemistries. Each chemistry is assigned a set of pipettes. Scroll to the top of the sheet and read the instructions carefully.

4.3. 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

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already entered. Always calibrate all volumes given in the spreadsheet. If any changes to the spreadsheet are needed contact your supervisor. You may have to scroll to the right side of the page to see all the entry fields. 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, which is located next to the balance.

Note: When using variable volume pipettes the accuracy at the maximum, middle and minimum values are checked. If only one value is calibrated the use of the pipette is restricted to this particular volume. The volume must be documented on the pipette label (see 4.7).

4.4. Check the calibration of the balance to be used (see DEP SOP CM-011). Bracket checks around expected values.

4.5. 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)”.

4.6. 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 5.

4.7. After the calibration is completed a LIMS generated label must be printed and attached to the pipette. 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 separate window opens. In this new window, under the “Options” menu, select “Four Lines of Text”. 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 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 chemistry 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:

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PIP ID 2301847
CAL DATE 11/10/11
250 uL only
PO4 PG

PIP ID 3992086
CAL DATE 12/20/11
TKN/TP analysis
CAA

5. QUALITY CONTROL AND CORRECTIVE ACTION

5.1. 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. Activate the "Repeat Calibration" tab of the worksheet. The left frame of this sheet is clearly marked in red. Perform a new calibration as described in section 4. 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.

5.2. In case the calibration fails again perform further maintenance or re-adjustments on the pipette. Activate the "Corrective Action" tab of the calibration worksheet. The left frame of this sheet is clearly marked in pink. Use this tab to calibrate the pipette for the third time following the procedure outlined in section 4. After the calibration is completed a comment must be entered in the "Comments" column of the spreadsheet detailing the corrective action performed. Notify your supervisor if a pipette could not be calibrated successfully. It must be taken out of service.

5.3. 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 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.

5.4. Do not use any other template for performing the monthly pipette calibrations. The monthly pipette calibrations are due by the 20th of each month. 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 month to correct any problems that may be observed at that time.

5.5. Hand-written pipette labels are not acceptable. Please refer to section 4.7 for details.

5.6. After the calibration worksheet is approved by the supervisor it is converted to pdf format for archival on the chemistry drive at \\FLDEP1\DEAR\Labs\Lab5\Chemistry\Inorganic\Nutrient\Pipette calibration\.

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6. 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

7. REFERENCES

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

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

Appendix of Significant Changes

01/03/2011 Section 4 was extensively modified, including instructions on the use of the new pipette calibration worksheets, to describe the calibration procedure in more detail. Section 5 was added to the SOP.

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