CALIBRATION OF AUTO-PIPETTES AND DISPENSERS USING THE CHECK & TRACK SOFTWARE SYSTEM

TABLE OF CONTENTS:

Page

1. SCOPE AND APPLICATION 2
2. SUMMARY OF THE METHOD 2
3. APPARATUS, EQUIPMENT AND SUPPLIES 2
4. PROCEDURE AND DOCUMENTATION 2
5. QUALITY CONTROL AND CORRECTIVE ACTION 9
6. CALCULATION 10
7. REFERENCES 11

APPENDIX OF SIGNIFICANT CHANGES 11

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1. SCOPE AND APPLICATION

1.1. This SOP describes the use of the Check & Track pipette calibration software in combination with the Mettler Toledo XPE105DR analytical balance to calibrate laboratory pipettes and dispensers. Pipettes must be calibrated at least quarterly, but may be calibrated on a more frequent basis to maintain the accuracy of analytical procedures.

2. SUMMARY OF THE METHOD

2.1. Pipettes and dispensers are calibrated using the known density of water. Aliquots of water are pipetted and weighed. The density of water is used to calculate the actual volume pipetted and this is compared to the expected volume. The density of water is dependent on atmospheric conditions: temperature, atmospheric pressure, and relative humidity. This dependency is expressed via a conversion factor called the "Z-factor" which relates the actual volume pipetted to its weighed value. The weighed value is first converted to true mass by applying the air buoyancy factor. The mass is then converted to volume by dividing it by the density of water. The accuracy (% difference) of the mean volumes is checked and the precision (% RSD) of replicate measurements are evaluated.

2.2. The Check & Track pipette calibration software is used in conjunction with the Mettler Toledo XPE105DR analytical balance to calibrate pipettes and dispensers. The software is locally installed on a computer (LDS2250) which is connected to the analytical balance via a serial cable for automatic data transfer. Pipettes, pipette tips, pipette checking methods, operators, balances, etc. are configured in the software by an administrator.

3. APPARATUS, EQUIPMENT AND SUPPLIES

3.1. Mettler Toledo XPE105DR analytical balance (accurate to 0.1 mg/0.01 mg dual range, maximum capacity: 120 g)

3.2. Check & Track pipette calibration and label printer software

3.3. Pipettes and pipette tips

3.4. Weighing vessels (glass tubes of various sizes)

3.5. Deionized (DI) water

3.6. Calibrated thermometer

3.7. Traceable digital barometer

4. PROCEDURE AND DOCUMENTATION

4.1. All pipettes are assigned unique ID numbers, according to their designated serial number at time of manufacturing. These ID numbers are documented on the

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pipette labels and as "Inventory Numbers" in the Check&Track software. Each pipette is added to the software by an administrator. If you start using a new or different pipette, notify your supervisor so that the new pipette and an applicable calibration method (if none already exists) can be added in the software. Each pipette is also assigned an operator. The operator is the primary analyst for the pipette's chemistry.

Note: If you are no longer the primary analyst for the pipette's chemistry, please notify your supervisor so that the operator in the software can be updated.

4.2. The Mettler Toledo XPE105DR balance is used exclusively for pipette calibrations and working calibration weight verifications.

4.3. Each pipette is assigned a **Pipette checking method** during setup in the software by the administrator. Each checking method includes the method name, number of measurements per test volume, number of test volumes, and acceptance criteria. The method name shall include the maximum pipette volume and type of pipette, e.g. 100 µL Adjustable Volume or 10 mL Fixed Volume. The pipette checking method for each pipette can only be changed by an administrator. Contact your supervisor if any changes are necessary.

Note: Once a Pipette checking method is set up and used for calibration, it cannot be modified.

4.4. For adjustable pipettes or bottle-top dispensers, the calibration is checked at low, middle, and high volumes which bracket or equal the range of use. For fixed-volume pipettes, bottle-top dispensers, or adjustable pipettes that are only used at fixed values, the calibration is checked at the volume used.

4.5. Check the calibration of the balance (see DEP SOP CM-011) if the status LED light on the balance display terminal is green. Bracket balance weight checks around expected measured mass values. Ensure the balance is free of debris or water droplets that could cause static charge.

Note: The status LED light around the edges of the balance display terminal should display a green color; if it displays an orange color, then the balance needs internal adjustment. To do this, close the Check&Track software and remove all weights from the weighing pan. Then, press the **Adjust. int** icon on the balance display to initiate internal adjustment. Proceed with balance calibration check after successful internal adjustment.

Note: Closing the Check&Track software is very important prior to internal adjustment of the balance. If this is not done, it can disrupt communication between the balance and software and will not print the weights during pipette calibration. This forces the user to cancel their check and restart the computer.

4.6. Launch the Check&Track software and select "Switch department", i.e. group, if another department is active (see Figure 1). From the drop-down list, select your initials and enter your password, then click "Login" to switch department. Once logged in, select the pipette to be calibrated from the list shown in the software. Alternatively, scan the barcode on the pipette calibration label or type the pipette identity (ID) code in the "Filter by manufacturer, name, or inventory number" tab to select the pipette. The lower part portion of the software window (see "Selected

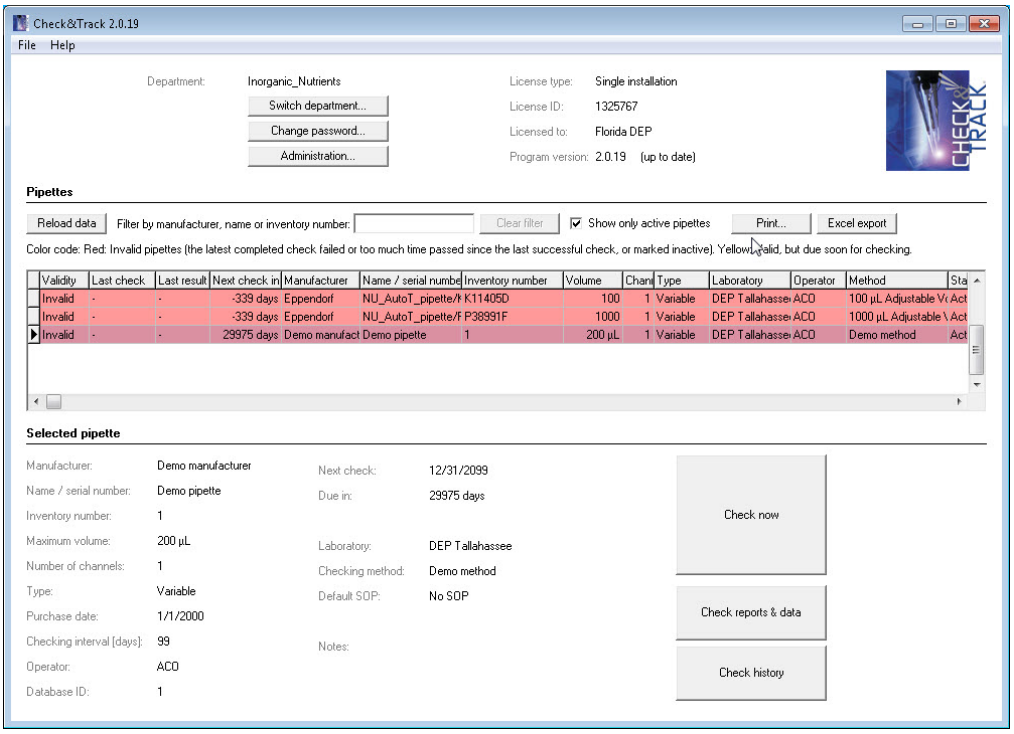
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pipette" in Figure 1) displays all the information about the selected pipette. Click "Check now" to start the calibration.

Note: The Pipette ID code is the unique identifier for each pipette.

4.7 Locate a weighing boat/vessel that can hold the volume of DI water to be pipetted. **Use only glass or metallic weighing vessels to reduce the effect of static charges.** Weigh the weighing vessel and tare the balance. The pipette and an appropriate pipette check method should already be set up in the software. Contact your supervisor if a pipette needs to be added to the software or if there is no appropriate method for the pipette.

Figure 1. Pipette Selection



4.8. In the **Pipette check** window that opens (see Figure 2 below), select the operator, enter the operator's password, select the pipette tips, enter the pipette tip lot number (optional), select the SOP and the balance. Additionally, enter the temperature of the water used for the calibration, atmospheric pressure, and relative humidity. The DI water should be in a flask with the thermometer, located next to the balance. The DI water should be replaced daily. The pressure and humidity readings are taken from the digital barometer. Once all the fields are filled in, click on **Start check now** to start the calibration.

4.9. In the next window that opens (see Figure 3 below), double-check and make sure the pipette ID and checking method match the pipette before starting the measurements. The volume to be measured is highlighted in the **Current Test Volume** line. If you are calibrating a variable volume pipette, make sure the pipette is set to the correct volume highlighted in the software. Pipette an aliquot

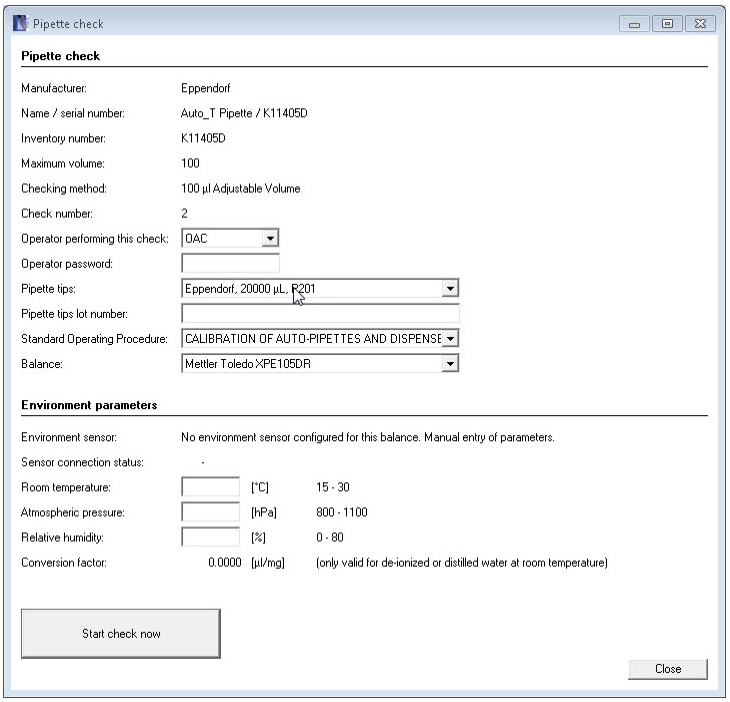
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of DI water into the vessel, wait until the reading is stable, press the **Print button** on the balance to transfer the measured data to the software. Tare the balance. Repeat the measurement and data transfer cycle the required number of times for each test volume.

Note: Each time the **Print** button is pressed, the value displayed on the balance is transferred to the software. **The "Print" button must be pressed only once for each measured data.** Ensure the value is displayed on the software prior to taring the balance.

Note: If the reading becomes unstable again immediately after pressing the print button, the software should automatically wait for stability before taking the weight. Do not press print again if you do not see the displayed value right away.

Figure 2. User login, pipette tip selection, and environmental parameters input



4.10. After each data transfer to the software, the next measurement number is highlighted in the **Current measurement** line (see Figure 3 below). After the fifth (5th) measured data point (i.e. the last measurement for a specified volume) is transferred, the **Next test volume** (for variable volume pipettes) appears. Click on the **Ok** button on the **Next test volume** pop-up window (see Figure 4 below) to continue.

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Figure 3. Pipette calibration measurements

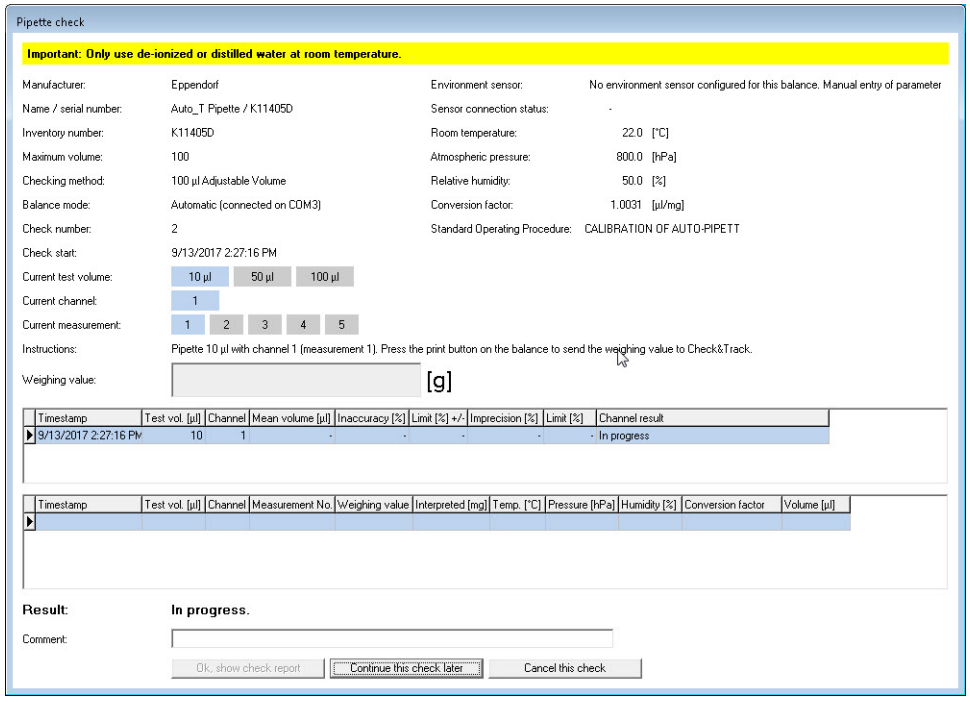
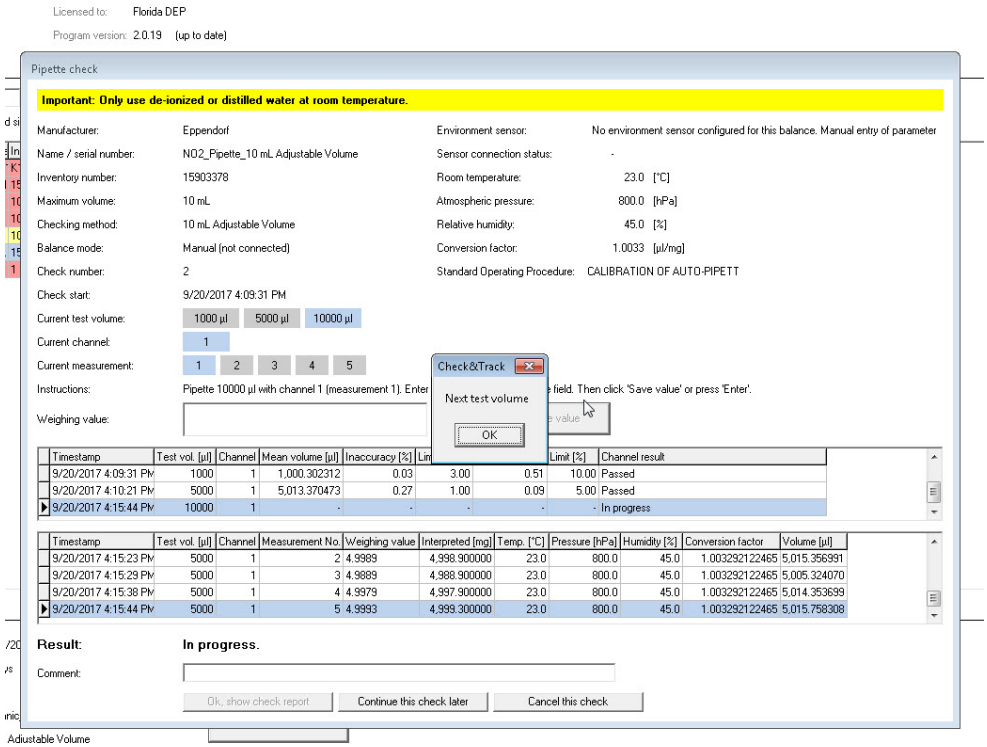


Figure 4. Pipette calibration measurements: Next test volume



4.11. The volume in µL for each aliquot is calculated automatically and recorded in the **Volume [µl]** column. The mean volume in µL and the acceptance criteria

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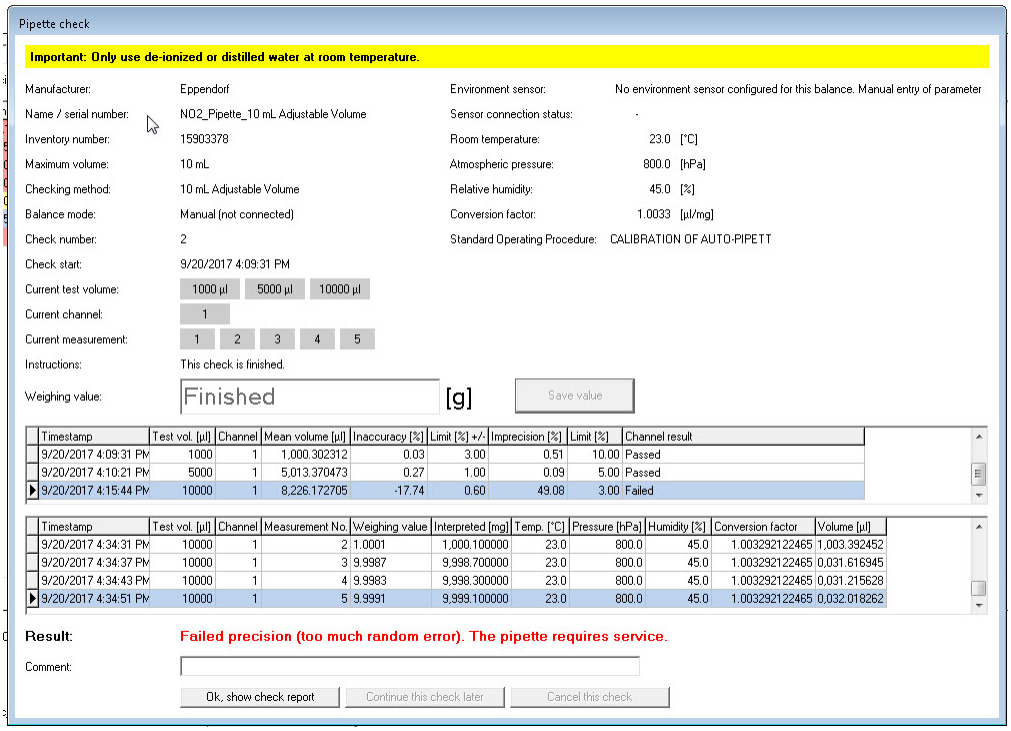
(accuracy and precision) for each checked volume are also calculated automatically and shown in the software (See Figure 4 above).

4.12. The pass/fail assessment for each calibrated volume is displayed in the **Channel result** column (see Figures 4 and 5). When the final measurement data is transferred, **Finished** is displayed on the **Weighing value** field in the software. Also, a summary result is displayed in the **Result** line at the lower part of the Pipette check window. The calibration fails if either accuracy or precision fails for any of the calibrated volumes. The explanation of the failure is shown in the result line. See Figure 5 below for an example.

4.13. Check the "Inaccuracy [%], "Imprecision [%]", "Channel result", and "Result:" fields (see Figure 5) to review the calibration. The inaccuracy must not be greater than the specifications set for the pipette. If the calibration passed, the **Result**: field will display **Passed**. If the calibration is not acceptable "**Failed** …" will be displayed along with a recommendation (See Figure 5). If a calibration fails, follow the corrective action procedure as described in Section 5.

Note: If a volume fails due to high inaccuracy or imprecision, the calibration can be cancelled (**Cancel this check**) rather than continued. A comment must be written when the check fails. (e.g. "Pipette will be adjusted and recalibrated before being put back into service.")

Figure 5. Example of pipette calibration result



Note: If only one value is calibrated, the use of the pipette is restricted to this volume. In this case, an appropriate fixed volume pipette checking method is assigned to the pipette by an administrator prior to pipette calibration. The volume must be documented on the pipette label (see 4.16).

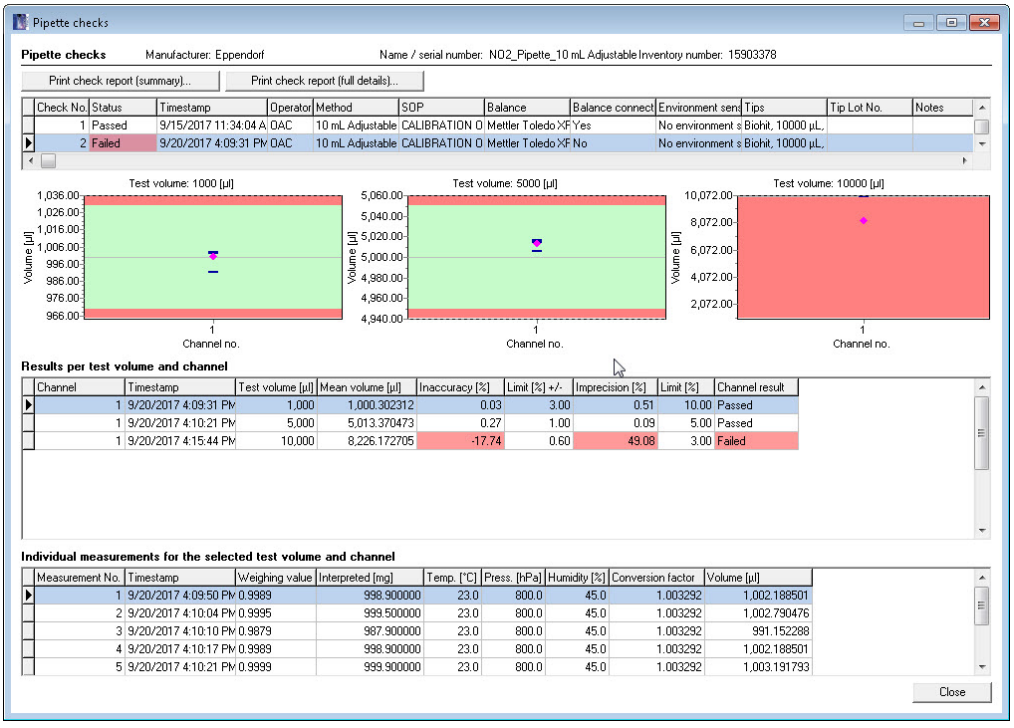
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4.14. Click **Ok, show check report** to exit the Pipette check window and view a summary report. To view a detailed report, close the summary report and click **Check reports & data** while the pipette is still selected (see Figure 1). In the window that opens (see Figure 6), click on **Print check report (full details)** to review the detailed calibration report.

4.15. If the calibration is acceptable, save the **Pipette Check Report (Full Details)** in the "**Pipette Calibration Certificates**" folder as a PDF document. This folder is located at \\floridadep\data\DEAR\Labs\Lab5\Chemistry\Pipette Calibration Certificates. Click on the print symbol, navigate to the current month's subfolder in the Inorganic_Metals or Inorganic_Nutrients folder, enter the file name to save the calibration file. The filename follows the format: Group_Analytical-Method_PipetteID_YYMMDD_Analyst-Initial, e.g. NU_TP_prep_D1583816T_181028_SF.

4.16. If a calibration is not acceptable, follow the corrective action procedure as described in Section 5. Do not save reports for failed calibrations in the reports folder, the raw data is automatically archived, and the report can be generated if needed. The raw data is archived on the network drive by the software.

Figure 6. Print check report (full details)

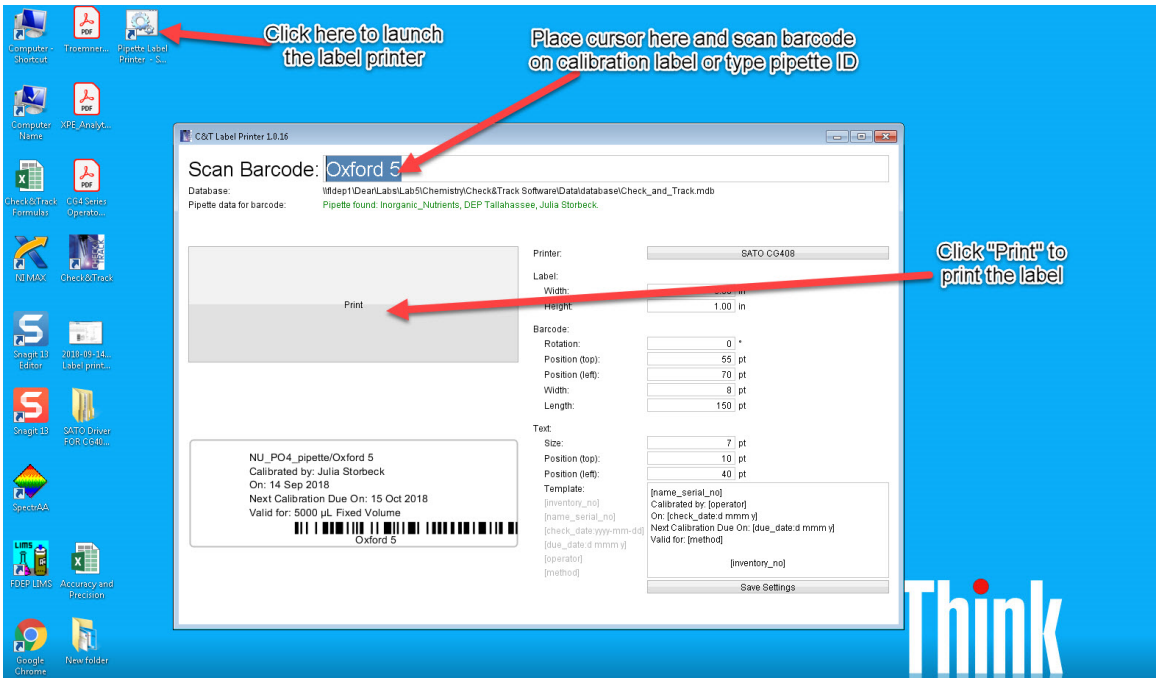


4.17. After the calibration is completed, a Check&Track generated calibration label with a barcode must be printed and attached to the pipette. Click on the Label Printer Shortcut on the computer desktop to launch the Check&Track label printer (see Figure 7 below). In the Scan Barcode field, scan the barcode on the pipette calibration label or type the pipette ID. The latest calibration data for the pipette will be automatically populated on the label preview. Click on the Print button to

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print the label. Attach the calibration label on the pipette with the barcode parallel to the long axis of the pipette so that the barcode scanner can scan it. The label contains the pipette name (includes group, chemistry, and pipette ID), name of analyst, calibration date, next calibration due date, and the calibration method used for the calibration.

Figure 7. Check&Track Label Printing



5. QUALITY CONTROL AND CORRECTIVE ACTION

5.1. Do not over-write calibration results. If a calibration result is not acceptable the software will display "**Failed**" in red in both the **Channel result** column and the "**Result:**" line of the pipette check window. In this case the pipette should be inspected for maintenance, adjustment, or repair, and the calibration must be repeated.

5.2. If the calibration failed and the pipette is subsequently adjusted, perform a new calibration as described in section 4. If the calibration passes at this point, add a "**Pipette adjusted and placed back in service**" comment in the **Comment** field in the pipette check window. The comment will show in the pipette calibration report.

5.3. If the calibration fails again, add "**Pipette cannot be adjusted, removed from service**" comment in the **Comment** field in the pipette check window. The comment will show in the pipette calibration report. Each pipette with a failed or expired calibration is highlighted in red in the software, while pipettes that are due for calibration within 7 days are highlighted in yellow. Notify your supervisor if a pipette could not be calibrated successfully. It must be repaired and re-calibrated

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or taken out of service. During review and repair, the failing pipette should be clearly labeled "**OUT OF SERVICE**".

5.4. All uncalibrated pipettes that are used in the lab for purposes like transferring concentrated acids or soaps/surfactants must be approved for the intended use by a supervisor. They must be clearly labeled "**NOT CALIBRATED**" and state the approved use.

5.5. A supervisor will check the calibration reports for correctness and completeness. The calibration reports files are saved in the **Pipette Calibration Certificates** folder, which is located in \\FLDEP1\DEAR\Labs\Lab5\Chemistry.

5.6. Hand-written pipette labels are normally not acceptable; however, they are permissible in case of label printer failure. Please refer to section 4.17 for details.

6. CALCULATION

6.1. The volume of the dispensed water is calculated by multiplying the weighed value by a correction factor called the "Z-factor". The weighed value is first converted to true mass by applying the air buoyancy factor. This mass is then converted to volume by dividing by the density of water. For more details please refer to the reference in 7.2.

$$V = Z * \text{weighed value}$$

where V is the volume of dispensed water (µL), Z is the Z-factor (µL/mg), and weighed value is the value displayed by the balance (mg).

The Z-factor is calculated by the following formula:

$$Z = \frac{1-\frac{\rho_{air}}{\rho_{conventional\ body}}}{1-\frac{\rho_{air}}{\rho_{water}}} * \frac{1}{\rho_{water}}$$

where ρ is density.

6.2. Accuracy (%): The software will calculate the percent difference (%D) between the nominal volume pipetted (Ve) and the calculated volume. The result is recorded in the **Inaccuracy [%]** column in the calibration software and the generated report.

$$\%D = (Va - Ve)/Ve * 100$$

where Ve = Volume of water according to pipette setting (µL),
Va = Average volume of dispensed water (µL) = ∑[Vn]/n
Vn = Volume of nth aliquot of water (µL)
n = Number of aliquots pipetted

6.3. Precision (%RSD): The precision is also calculated automatically in the software as the standard deviation of the replicate measurements in µL divided by the mean

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volume in µL. The result is recorded in the **Imprecision [%]** column in the calibration software and the generated report.

$$\%RSD = s/Va * 100$$

Where s = Standard deviation

7. REFERENCES

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

7.2. Check&Track, Determination of the pipette volume based on the mass of the dispensed water. Reto Höhener, 2017

Appendix of Significant Changes

12/10/2018 Updated Section 4 with the Check&Track label printer feature and instructions.

12/15/2022 File naming convention changed from Method_PipetteID_MMDDYY to Method_PipetteID_YYMMDD.

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