

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

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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

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

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

The screenshot shows the Check&Track 2.0.19 software window. At the top, there are fields for Department (Inorganic_Nutrients), License type (Single installation), License ID (1325767), Licensed to (Florida DEP), and Program version (2.0.19 up to date). Below this is the 'Pipettes' section with a table listing various pipettes. The table has columns for Validity, Last check, Last result, Next check in, Manufacturer, Name / serial number, Inventory number, Volume, Chan, Type, Laboratory, Operator, Method, and Status. The third row is highlighted in red, indicating it is invalid. Below the table is the 'Selected pipette' section, which displays details for the selected pipette (Demo manufacturer, Demo pipette, Inventory number: 1, Maximum volume: 200 µL, Number of channels: 1, Type: Variable, Purchase date: 1/1/2000, Checking interval [days]: 99, Operator: ACO, Database ID: 1). On the right side of this section are three buttons: 'Check now', 'Check reports & data', and 'Check history'.

Validity	Last check	Last result	Next check in	Manufacturer	Name / serial number	Inventory number	Volume	Chan	Type	Laboratory	Operator	Method	Sta
Invalid	-	-	:339 days	Eppendorf	NU_AutoT_pipette/f K11405D		100	1	Variable	DEP Tallahassee ACO		100 µL Adjustable Vt Act	
Invalid	-	-	:339 days	Eppendorf	NU_AutoT_pipette/f P38991F		1000	1	Variable	DEP Tallahassee ACO		1000 µL Adjustable Vt Act	
Invalid	-	-	29975 days	Demo manufact	Demo pipette	1	200 µL	1	Variable	DEP Tallahassee ACO		Demo method	Act

Selected pipette

Manufacturer: Demo manufacturer
Name / serial number: Demo pipette
Inventory number: 1
Maximum volume: 200 µL
Number of channels: 1
Type: Variable
Purchase date: 1/1/2000
Checking interval [days]: 99
Operator: ACO
Database ID: 1

Next check: 12/31/2099
Due in: 29975 days
Laboratory: DEP Tallahassee
Checking method: Demo method
Default SOP: No SOP
Notes:

Check now
Check reports & data
Check history

- 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

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

The screenshot shows a software window titled "Pipette check". It contains two main sections: "Pipette check" and "Environment parameters".

Pipette check section:

- Manufacturer: Eppendorf
- Name / serial number: Auto_T Pipette / K11405D
- Inventory number: K11405D
- Maximum volume: 100
- Checking method: 100 µl Adjustable Volume
- Check number: 2
- Operator performing this check: DAC (dropdown menu)
- Operator password: (text input field)
- Pipette tips: Eppendorf, 20000 µL, R201 (dropdown menu)
- Pipette tips lot number: (text input field)
- Standard Operating Procedure: CALIBRATION OF AUTO-PIPETTES AND DISPENSE (dropdown menu)
- Balance: Mettler Toledo XPE105DR (dropdown menu)

Environment parameters section:

- Environment sensor: No environment sensor configured for this balance. Manual entry of parameters.
- Sensor connection status: -
- Room temperature: (text input field) [°C] 15 - 30
- Atmospheric pressure: (text input field) [hPa] 800 - 1100
- Relative humidity: (text input field) [%] 0 - 80
- Conversion factor: 0.0000 [µl/mg] (only valid for de-ionized or distilled water at room temperature)

At the bottom of the window, there are two buttons: "Start check now" and "Close".

- 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.

Figure 3. Pipette calibration measurements

Pipette check

Important: Only use de-ionized or distilled water at room temperature.

Manufacturer: Eppendorf
Name / serial number: Auto_T Pipette / K11405D
Inventory number: K11405D
Maximum volume: 100
Checking method: 100 µl Adjustable Volume
Balance mode: Automatic (connected on COM3)
Check number: 2
Check start: 9/13/2017 2:27:16 PM
Current test volume: 10 µl 50 µl 100 µl
Current channel: 1
Current measurement: 1 2 3 4 5
Instructions: Pipette 10 µl with channel 1 (measurement 1). Press the print button on the balance to send the weighing value to Check&Track.
Weighing value: [g]

Environment sensor: No environment sensor configured for this balance. Manual entry of parameter
Sensor connection status: -
Room temperature: 22.0 [°C]
Atmospheric pressure: 800.0 [hPa]
Relative humidity: 50.0 [%]
Conversion factor: 1.0031 [µl/mg]
Standard Operating Procedure: CALIBRATION OF AUTO-PIPETT

Timestamp	Test vol. [µl]	Channel	Mean volume [µl]	Inaccuracy [%]	Limit [%] +/-	Imprecision [%]	Limit [%]	Channel result
9/13/2017 2:27:16 PM	10	1	-	-	-	-	-	In progress

Timestamp	Test vol. [µl]	Channel	Measurement No.	Weighing value	Interpreted [mg]	Temp. [°C]	Pressure [hPa]	Humidity [%]	Conversion factor	Volume [µl]
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Result: In progress.
Comment:

Ok, show check report Continue this check later Cancel this check

Figure 4. Pipette calibration measurements: Next test volume

Licensed to: Florida DEP
Program version: 2.0.19 (up to date)

Pipette check

Important: Only use de-ionized or distilled water at room temperature.

Manufacturer: Eppendorf
Name / serial number: NO2_Pipette_10 mL Adjustable Volume
Inventory number: 15903378
Maximum volume: 10 mL
Checking method: 10 mL Adjustable Volume
Balance mode: Manual (not connected)
Check number: 2
Check start: 9/20/2017 4:09:31 PM
Current test volume: 1000 µl 5000 µl 10000 µl
Current channel: 1
Current measurement: 1 2 3 4 5
Instructions: Pipette 10000 µl with channel 1 (measurement 1). Enter
Weighing value:

Environment sensor: No environment sensor configured for this balance. Manual entry of parameter
Sensor connection status: -
Room temperature: 23.0 [°C]
Atmospheric pressure: 800.0 [hPa]
Relative humidity: 45.0 [%]
Conversion factor: 1.0033 [µl/mg]
Standard Operating Procedure: CALIBRATION OF AUTO-PIPETT

Check&Track
Next test volume
OK

Timestamp	Test vol. [µl]	Channel	Mean volume [µl]	Inaccuracy [%]	Limit [%]	Channel result
9/20/2017 4:09:31 PM	1000	1	1,000.302312	0.03	3.00	10.00 Passed
9/20/2017 4:10:21 PM	5000	1	5,013.370473	0.27	1.00	5.00 Passed
9/20/2017 4:15:44 PM	10000	1	-	-	-	In progress

Timestamp	Test vol. [µl]	Channel	Measurement No.	Weighing value	Interpreted [mg]	Temp. [°C]	Pressure [hPa]	Humidity [%]	Conversion factor	Volume [µl]
9/20/2017 4:15:23 PM	5000	1	2	4.9989	4,998.900000	23.0	800.0	45.0	1.003292122465	5,015.356991
9/20/2017 4:15:29 PM	5000	1	3	4.9889	4,988.900000	23.0	800.0	45.0	1.003292122465	5,005.324070
9/20/2017 4:15:38 PM	5000	1	4	4.9979	4,997.900000	23.0	800.0	45.0	1.003292122465	5,014.353699
9/20/2017 4:15:44 PM	5000	1	5	4.9993	4,999.300000	23.0	800.0	45.0	1.003292122465	5,015.758308

Result: In progress.
Comment:

Ok, show check report Continue this check later Cancel this check

Adjustable 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

(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

Pipette check

Important: Only use de-ionized or distilled water at room temperature.

Manufacturer:	Eppendorf	Environment sensor:	No environment sensor configured for this balance. Manual entry of parameter
Name / serial number:	ND2_Pipette_10 mL Adjustable Volume	Sensor connection status:	-
Inventory number:	15903378	Room temperature:	23.0 [°C]
Maximum volume:	10 mL	Atmospheric pressure:	800.0 [hPa]
Checking method:	10 mL Adjustable Volume	Relative humidity:	45.0 [%]
Balance mode:	Manual (not connected)	Conversion factor:	1.0033 [µl/mg]
Check number:	2	Standard Operating Procedure:	CALIBRATION OF AUTO-PIPETT
Check start:	9/20/2017 4:09:31 PM		
Current test volume:	1000 µl 5000 µl 10000 µl		
Current channel:	1		
Current measurement:	1 2 3 4 5		
Instructions:	This check is finished.		
Weighing value:	Finished [g]		Save value

Timestamp	Test vol. [µl]	Channel	Mean volume [µl]	Inaccuracy [%]	Limit [%] +/-	Imprecision [%]	Limit [%]	Channel result
9/20/2017 4:09:31 PM	1000	1	1,000.302312	0.03	3.00	0.51	10.00	Passed
9/20/2017 4:10:21 PM	5000	1	5,013.370473	0.27	1.00	0.09	5.00	Passed
9/20/2017 4:15:44 PM	10000	1	8,226.172705	-17.74	0.60	49.08	3.00	Failed

Timestamp	Test vol. [µl]	Channel	Measurement No.	Weighing value	Interpreted [mg]	Temp. [°C]	Pressure [hPa]	Humidity [%]	Conversion factor	Volume [µl]
9/20/2017 4:34:31 PM	10000	1	2	1.0001	1,000.100000	23.0	800.0	45.0	1.003292122465	1,003.392452
9/20/2017 4:34:37 PM	10000	1	3	9.9987	9,998.700000	23.0	800.0	45.0	1.003292122465	0,031.616945
9/20/2017 4:34:43 PM	10000	1	4	9.9983	9,998.300000	23.0	800.0	45.0	1.003292122465	0,031.215628
9/20/2017 4:34:51 PM	10000	1	5	9.9991	9,999.100000	23.0	800.0	45.0	1.003292122465	0,032.018262

Result: Failed precision (too much random error). The pipette requires service.

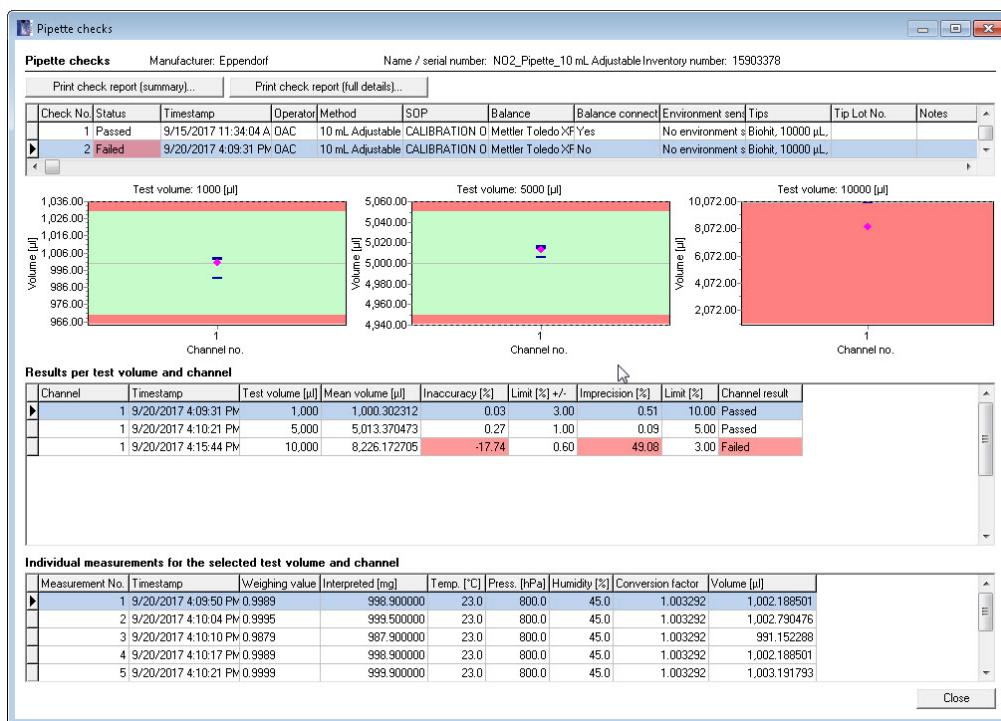
Comment:

Ok, show check report Continue this check later Cancel this check

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).

- 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.

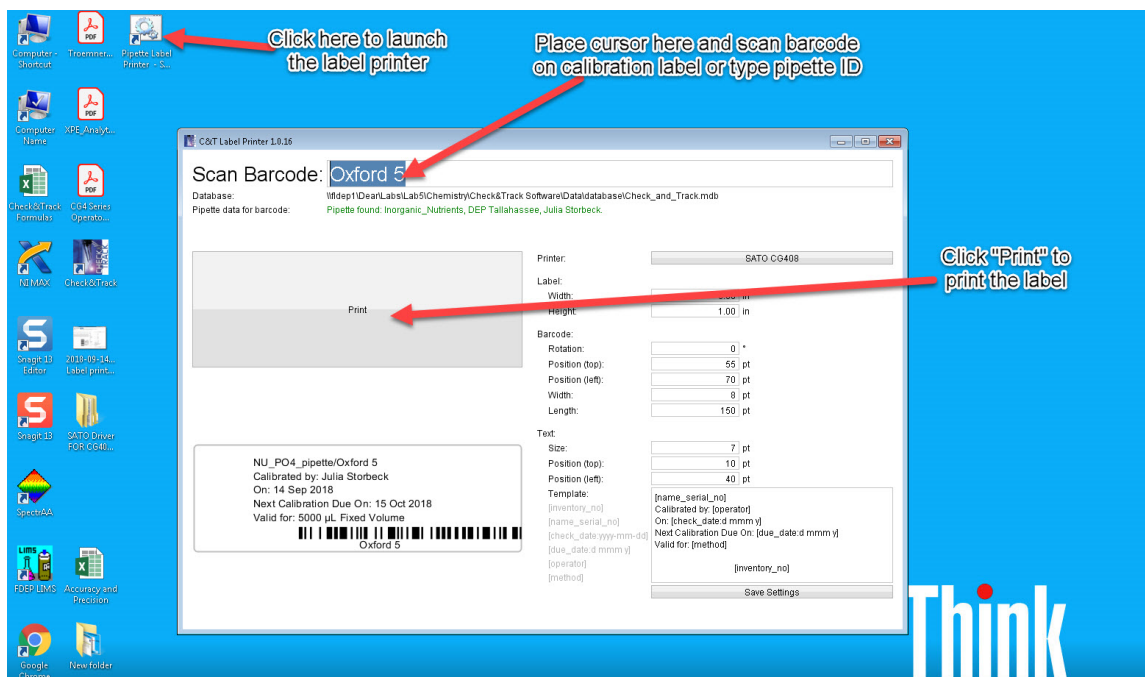
Figure 6. Print check report (full details)



- 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

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

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:

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

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.

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

where Ve = Volume of water according to pipette setting (μL),
Va = Average volume of dispensed water (μL) = $\sum[V_n]/n$
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

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.