# Calibration And Use of Mechanical Pipettes Using the Sartorius Cubis® II Balance

## 1. SCOPE AND APPLICATION

1.1. To maintain the accuracy and precision of analytical procedures, proper pipetting techniques should be followed. For each mechanical pipette, calibrate at **three** volume levels (low, mid, high) that bracket the intended range, with **≥ 10 replicate measurements** per level. If a mechanical pipette is used at only one volume, verification at that single volume is sufficient. This calibration procedure is based on the Sartorius Ingenix® Suite Operating Instructions and applies to mechanical pipettes ranging from 0.5 to 10,000 µL.

## 2. SUMMARY OF METHOD

2.1. Pipettes are calibrated gravimetrically using a Sartorius Cubis® II balance configured to the Sartorius Ingenix® Suite software. A predetermined volume of deionized water is pipetted into the moisture trap. Once the draft shield is closed, the balance stabilizes, and the measurement is recorded in the software. At least ten measurements are made per calibration volume. Gravimetric measurements are corrected for air buoyancy using ambient conditions (temperature, humidity, and barometric pressure), which are manually entered into the software before calibration. The Ingenix® software applies ISO 8655-6:2022 Formula 2 for conversion of weighing value to volume ) to calculate traceable and accurate pipette performance.

2.2. Balance Features:

2.2.1. Use **white side buttons** on the display to open/close the draft shield. These buttons can be programmed to open part or all of the draft shield. **Do not operate it manually as it increases wear and tear on the internal mechanisms**.

2.2.2. The balance is equipped with a built-in ionizer. Within the draft shield along the back wall of the balance, there are four inserts that represent the ionizer. This ensures that any electrostatic charges are removed from samples. The ionizer functions automatically and does not need to be prompted.

2.3. Interferences:

2.3.1. Temperature. Allow pipettes and water to equilibrate to ambient temperature for approximately 2 hours prior to pipetting.

2.3.2. Humidity. Extreme conditions can affect precision and accuracy by increased rate of evaporation or condensation.

2.3.3. Vibrations. Avoid using balance while external vibrations (i.e. centrifuges) or pressing on the balance table during calibration as this may cause unstable readings.

2.3.4. Electrostatic Charge. Handled by the ionizer; ensure the ionizer is functional before each session.

2.4. Environmental Conditions



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2.4.1. Calibration must be performed under stable and controlled environmental conditions. These conditions are based on both ISO 8655-6/7 and Sartorius manufacturer recommendations to ensure the accuracy of gravimetric measurements.

2.4.1.1. Draft free and stable environment

2.4.1.2. Relative Humidity: **20% - 80%**

2.4.1.3. Temperature: **15°C** to **27°C**

2.4.1.4. Temperature variation during calibration must not exceed **±0.5 °C** per hour

**Note:** While ISO 8655 recommends a working temperature of 20 ± 3 °C, the stability of the temperature during the measurement period is more critical than the exact value. Formula 2remains valid within the temperature, humidity, and pressure ranges listed above and is applied by the software based on the manually entered parameters. Contact a supervisor if room conditions fall outside the specified range. Calibration should not proceed unless approved by supervisor and properly documented. Additionally, if the instrument is unplugged, it must be allowed to stabilize for at least 24 hours before use to ensure it is fully acclimated to the environment.

## 3. EQUIPMENT AND SUPPLIES

3.1. Sartorius Cubis® II MCA66S-3S00-D Balance (S/N 0046605431)

3.2. Sartorius Ingenix® Suite V.2.3.0

3.3. Sartorius Moisture trap for Cubis® II

3.4. Mechanical Pipettes – See Table 12.1

3.5. Deionized water for pipetting

3.6. NIST traceable digital thermometer

3.7. NIST traceable digital barometer/hygrometer

3.8. Calibration weights (Class 2 working weights and Chemistry (Class 1 or 0) reference weights)

3.9. KimWipes

3.10. Gloves, latex or nitrile

## 4. PROPER PIPETTING TECHNIQUES

4.1. Adjustable and Fixed Volume Pipettes

4.1.1. Check the volume setting before use. Adjust from a higher to a lower volume and store the pipette at the highest setting of its range to reduce spring stress and prolong lifespan.



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4.2. To ensure accurate and consistent delivery across all volumes, **pre-wet the pipette tip exactly three times** by aspirating and dispensing the intended volume before beginning measurements. This is especially critical for smaller volumes where evaporation and tip conditioning have a greater impact on accuracy.

4.3. Release the control button to the first stop, hold the pipette vertically, and immerse the tip only a few millimeters into the liquid to avoid external carryover and aspiration error.

4.4. Release the control button slowly and evenly keeping the pipette vertical; holding it at an angle reduces accuracy. Wait one to three seconds (5 seconds for 1-10 mL pipettes) before lifting the tip straight out of the liquid. If a droplet remains outside of the tip, gently wipe it against the container wall.

4.4.1. Wear gloves, hold the pipette loosely, and set it down when not actively pipetting to prevent body heat from affecting the pipette.

4.4.2. Maintain consistent pressure and a steady pipetting speed throughout the aspiration and dispensing steps to promote precision.

4.5. Adjustable Volume Repeater Pipettes

4.5.1. Slide the filling lever all the way to the bottom and hold it there; then insert the tip from below while keeping the lever down.

4.5.2. Rotate the volume-selection wheel to the appropriate volume until it clicks in the desired setting and locks in place.

4.5.3. Immerse the pipette tip in the liquid. Push the filling lever up slowly and uniformly until it reaches the stop.

4.5.4. Prime the pipette by placing the tip on the tube wall and pressing the dispensing lever once; this brings the system to the baseline position.

4.5.5. To dispense, place the tip against the wall of the tube and push the dispensing lever down to the stop, listening for the characteristic gear click that confirms a complete discharge. If the click is absent, the tip lacks sufficient volume and must be refilled following the earlier steps.

4.5.6. After dispensing, leave the tip in the original liquid vessel and push the filling lever to the bottom to expel any remaining liquid.

4.5.7. Eject the used tip by holding the pipette over a waste container and pressing both ejection keys simultaneously.

## 5. ASSIGNING USER ROLES IN THE SARTORIUS INGENIX® SUITE SOFTWARE V.2.3.0

5.1. An administrator must log in to the PC adjacent to the Cubis II® balance using their DEP Windows credentials and launch the Sartorius Ingenix® Suite software. Once logged in, the administrator may assign roles to analysts by navigating to **System > User Management** and accessing the **Edit Role** module. Each role is associated with



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specific privileges, organized into four main permission categories, each containing multiple configurable options. Refer to Figure 1: Permission Categories for details.

5.2. Permission categories are as follows:

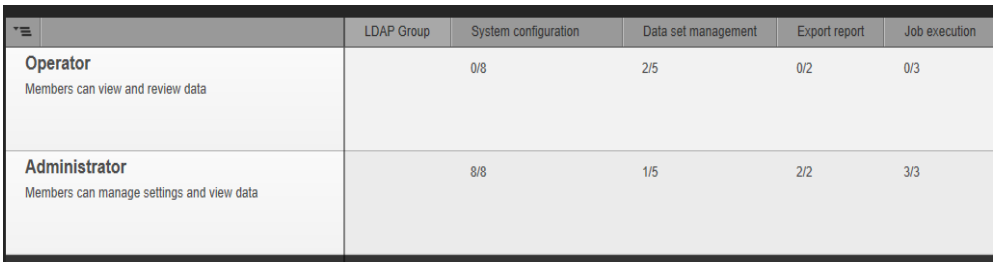
5.2.1. System Configuration

5.2.2. Data Set Management

5.2.3. Export Job

5.2.4. Execution Job.

Figure 1: Permission Categories

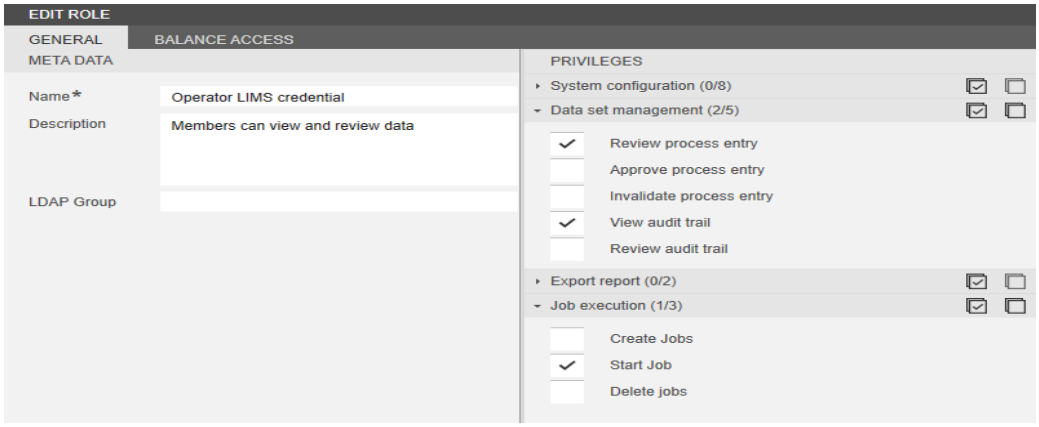


| | LDAP Group | System configuration | Data set management | Export report | Job execution |
|---|---|---|---|---|---|
| **Operator** Members can view and review data | | 0/8 | 2/5 | 0/2 | 0/3 |
| **Administrator** Members can manage settings and view data | | 8/8 | 1/5 | 2/2 | 3/3 |

5.3. When creating a new role, enter the analyst's LIMS credential using the format: RoleName LastName_FirstInitial (e.g., Operator SMITH_J). Refer to Figure 2: Role privileges for details.

5.4. All analysts and managers must log in to the PC adjacent to the Cubis II® balance using their DEP Windows credentials. The software uses these credentials to record audit trails and capture calibration signatures.

Figure 2: Role Privileges






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# 6. ADDING EQUIPMENT INTO THE SARTORIUS INGENIX® SUITE SOFTWARE V.2.3.0

6.1. Adding Pipettes:

6.1.1. Open the Ingenix® Suite Software V.2.3.0 and log in as an administrator.

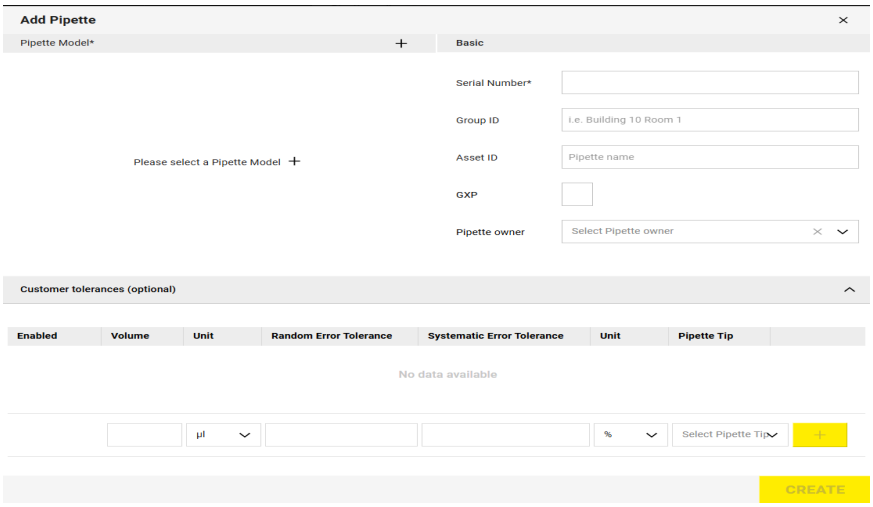
6.2. Navigate to the *Pipette Calibration* module and select *Device Management*.

6.3. Under the Pipette Model tab, click the “Add” (+) button. Search for and select the appropriate pipette model from the default list. Refer to Figure 3: Pipette Information Module for details.

**Note**: If the model is already listed, a new entry is not required.

6.4. Still under Device Management, select the Pipettes menu and click “Add” (+) button to enter the following information: Serial Number, Group ID, Asset ID, and Tolerances as listed in the Pipette Master Sheet, available in the SARTORIUS Folder.

Figure 3: Pipette Information Module




6.4.1. Click on the CREATE button to add the pipette to the table.

6.4.2. Navigate to Device Management and then to Pipette Tips to add the tips associated with the new pipette if an entry does not already exist.

6.5. Adding Configurations:

6.5.1. Open the Ingenix® Suite Software V.2.3.0 and log in as an administrator.

6.6. Navigate to the Pipette Calibration module and select Configurations. Refer to Figures 4 and 5 for details.

6.7. Click “Add” to input pipette calibration information.

6.8. Define the name of the configuration, set the schedule, and set the tolerance type to Customer.

6.9. Require a new tip for each calibration point



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6.10. Require digital signing (Windows password) before a calibration report can be finalized

**Note:** Use "Customer" tolerances and ensure proper error hierarchy. Tolerances must match the Master Sheet and follow software rules.

Figure 4: Configurations

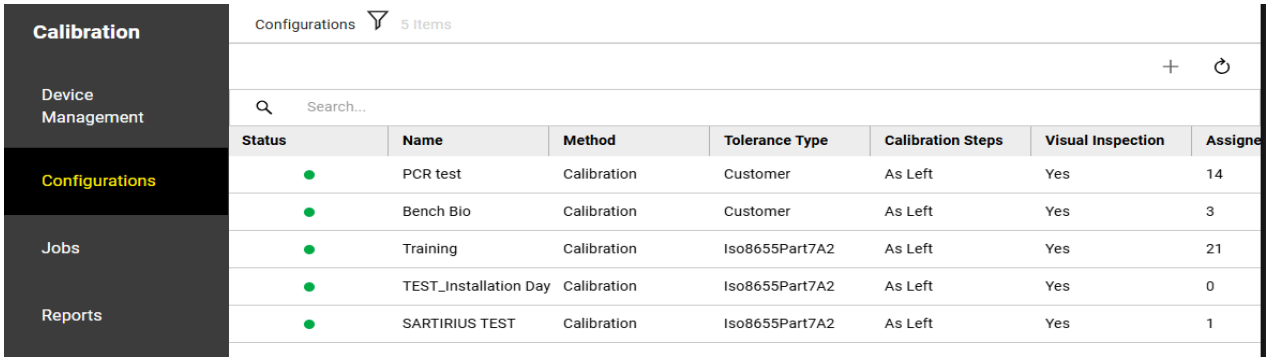
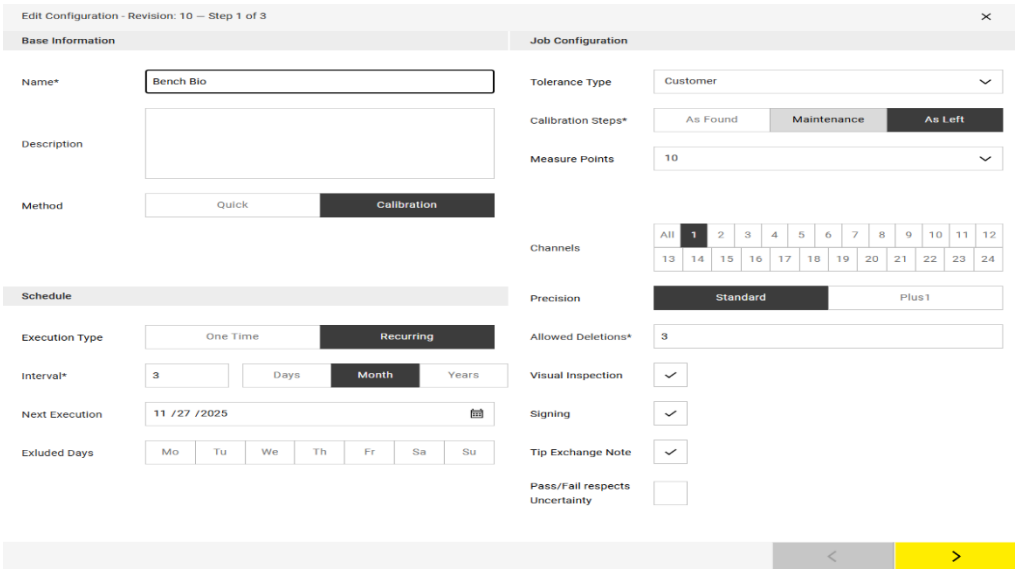



Figure 5: Configurations Requirements




6.11. Adding Jobs:

6.11.1 To create a job, the pipette and configuration must already have been added to the software. Only after a job has been created can pipette calibrations proceed.

6.11.2 Open the Ingenix® Suite Software V.2.3.0 and log in as an administrator.

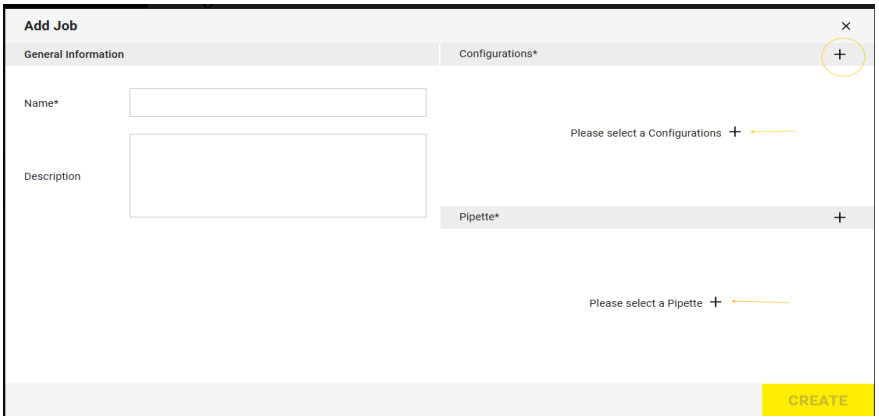
6.11.3 Navigate to the Pipette Calibration module and select Jobs. Refer to Figure 6: Job Module for details.



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6.11.4 Click “Add” to start a new job and select the appropriate configuration and pipette; then click on the CREATE button to add the job to the table.

Figure 6: Job Module




## 7. CALIBRATION AND USE OF CUBIS II® BALANCE

7.1. Ensure the weighing pan is unloaded. Turn the balance on by logging in on the display screen. The balance has an internal motorized level therefore no manual leveling is required.

7.2. For daily balance calibration verification, weigh Class II weights that bracket range of use. Record weights in the calibration logbook.

7.3. For annual balance calibrations, weigh and record all Class 1 reference weights. Refer to SOP CM-011 Gravimetric Analysis: Analytical Weight and Balance Calibration. Notify a supervisor if the Class 1 weights are not within tolerances found in the Calibration log. An outside weighing service provider annually calibrates all balances.

## 8. CALIBRATION AND DOCUMENTATION OF MECHANICAL PIPETTES

8.1. On the **first of each month**, review the **Pipette Status List** in the *Pipette Calibration* module under the **Status** menu to identify upcoming calibration due dates.

8.2. Preparation

8.2.1. Follow the steps in Section 7 to bracket the range of weights for use.

8.2.2. Ensure the DI water, pipettes, and tips used for calibration have acclimated to room temperature before use. (approximately 2 hours).

8.3. Log in on the PC with personal DEP Windows account. In the software on the PC, navigate to the *Pipette Calibration* module and click on *Jobs*. Open the *Pipette Calibration* module → **Jobs** → select a job → click **Start**.

8.4. On the balance select the **Weighing** application.



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8.5. Visually inspect the pipette and confirm that it is INTACT when prompted on the screen. Select DEFECT if leaks or damage are observed.

8.6. Measure and record the temperature of the DI water in the moisture trap. Record the thermometer serial number in the software.

8.7. Measure and record the temperature of the DI water in the moisture trap using a NIST-traceable thermometer. Record the thermometer's serial number in the software.

8.8. Measure and record ambient conditions (air temperature, pressure, and humidity) using a NIST- traceable barometer/hygrometer. Record the barometer/hygrometer serial number in the software.

8.9. Calibration Steps.

8.9.1. Make sure **Automatic Measurements** is toggled **ON**. Tare the balance by selecting the **"T"** button on the balance display screen.

8.9.2. Set the pipette to the volume displayed in the software.

8.9.3. When prompted the message **[Please pipette]**, pipette the test volume into the moisture trap inside the balance. Close the draft shield and wait for the measurement to display. The balance will automatically tare before the next pipetting step.

**Note:** Always use the side buttons to open and close the draft shields. Avoid opening or closing them manually to prevent unnecessary wear and tear.

8.9.4. If Automatic Measurements are off, the system defaults to manual acceptance. In this mode, click the ACCEPT button once the measuring point appears.

8.9.5. Repeat until all test measurements are completed.

8.9.6. The maximum weight on the balance is 61 g, including the tared vessel. The moisture trap has a total volume capacity of 21 mL. When the water level approaches 15 mL, carefully transfer the excess into a designated DI water beaker (do not discard). This allows the water to remain acclimated and ready for continued use. The balance will automatically tare between readings.

8.10. Post-Calibration Steps.

8.10.1. Record final ambient conditions again (air, water temperature, pressure, humidity) and click NEXT. Record the serial numbers of the barometer/hygrometer and temperature probe.

8.10.2. On the preview page of the report, enter your personal Windows password and provide a signature if prompted. Click CLOSE to finish



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8.10.3. Record the calibration date, analyst initials, and next calibration due date. The software does not currently have a label printing feature. Labels can be created using the **Print Prep Labels** module under **Prep Worksheet Manager** in **LIMS**.

## 9. DATA ARCHIVAL

9.1. Locate all calibration files under the Reports menu.

9.2. All calibration files should be automatically exported and stored in .pdf format.

9.3. All files should be saved in the appropriately dated folder in the following directory: \\floridadep\Data\DEAR\Labs\Lab3\Biology\SARTORIUS

9.4. All calibration files should have the following naming convention which includes the pipette serial number, date, and initials of the pipette calibrator.

9.5. File name: Serial number-YYMMDD-FL.pdf

## 10. QUALITY CONTROL AND CORRECTIVE ACTION

10.1. If a measured point needs to be re-read due to user error, on the pipette calibration screen, select the DELETE button and an Undo Reason from the drop-down menu. Once the measurement is deleted it will need to be determined again. The number of deletions available is reduced. Each pipette calibration job has a maximum of 3 allowed deletions. If errors were not observed when pipetting, do not delete any outlier points.

10.2. If a pipette fails a calibration run, save the calibration report as outlined in Section 9 and notify a supervisor. If the failure appears to be due to user error, a repeat calibration may be performed. Refer to Section 4 for proper pipetting techniques. If the pipette fails a second time, inspect it for leaks, damage, or cleanliness (see Section 11 for maintenance guidance). If adjustment is needed, follow the manufacturer's instructions to make the necessary corrections, then perform another calibration verification.

10.3. 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 must be approved for the intended use by the supervisor. They must be clearly labeled "NOT CALIBRATED" and state the approved use.

10.4. 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 about the same for each weighing, then the pipette may need an adjustment. If precision fails, make sure to use proper pipetting techniques consistently. Precision cannot be adjusted on the pipette. Precision is mostly affected



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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.

## 11. MAINTENANCE

11.1. Pipette Maintenance

11.2. Cleaning: Before use, always check the outside of the pipette for cleanliness. 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 deionized water only. Remove any old grease before lubricating the piston sealing rings.

11.3. 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. Test the pipette calibration after any adjustments are made and document the adjustment in the calibration report.

11.4. Balance Maintenance

11.4.1. Select the cleaning application on the balance display screen. The application will guide the user through step-by-step cleaning process of the balance.

11.4.2. The balance can be wiped clean with a Kim Wipe and deionized water or 70% ethanol.

11.5. Moisture Trap Maintenance

11.5.1. Inspect the moisture trap regularly and empty excess water as needed to prevent overflow. To clean, remove the moisture trap according to the manufacturer's instructions. Rinse with deionized water and dry thoroughly with a KimWipe before reinstalling.

## 12. TABLES

12.1. Pipettes Calibrated with Sartorius Ingenix® Suite Software

| Pipette ID | Volume (µL) | Type | Group |
|---|---|---|---|
| 1765876 | 10-100 | Eppendorf Reference | Bench Bio |
| L40124J | 100-1000 | Eppendorf Research Plus | Bench Bio |
| P14666N | 100-1000 | Eppendorf Research Plus | Bench Bio |
| R20289F | 100 | Eppendorf Reference 2 | Bench Bio |
| K45340 J | 0.5-5000 | Eppendorf Research Plus | Bench Bio |
| I21192N | 0.5-5000 | Eppendorf Research Plus | Bench Bio |
| EH75658 | 2000-10000 | Finnipipette II | Bench Bio |
| I57847H | 12500 | Eppendorf Repeater E3 | Bench Bio |
| P58802L | 0.5-10 | Eppendorf Research Plus | Molecular |
| P58890L | 0.5-10 | Eppendorf Research Plus | Molecular |
| P81287K | 0.5-10 | Eppendorf XplorerPlus | Molecular |
| K39700L | 0.5-10 | Eppendorf XplorerPlus | Molecular |
| L14911B | 2-20 | Eppendorf Research Plus | Molecular |
| L15097B | 2-20 | Eppendorf Research Plus | Molecular |



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| | | | |
|---|---|---|---|
| I36523H | 2-20 | Eppendorf Research Plus | Molecular |
| J18706B | 10-100 | Eppendorf Research Plus | Molecular |
| M24765B | 20-200 | Eppendorf Research Plus | Molecular |
| M24817B | 20-200 | Eppendorf Research Plus | Molecular |
| I32865B | 100-1000 | Eppendorf Research Plus | Molecular |
| I32903B | 100-1000 | Eppendorf Research Plus | Molecular |
| I32941B | 100-1000 | Eppendorf Research Plus | Molecular |
| I32943B | 100-1000 | Eppendorf Research Plus | Molecular |

## 13. REFERENCES

13.1. Ingenix® Suite Operating Instructions Manual

13.2. ISO-8655-6-2022 Piston-operated volumetric apparatus- Part 6: Gravimetric reference measurement procedure for the determination of volume: \\floridadep\data\DEAR\Labs\Lab5\Biology\BenchCommon\Methods & Library Files\ISO methods

13.3. Eppendorf Multipipette plus/Repeater plus/Xplorer plus Operating Manual

13.4. 8 Easy Steps for Performing Pipette Preventive Maintenance: \\floridadep\Data\DEAR\Labs\Lab5\Biology\BenchCommon\Equipment

13.5. Pipette Master Sheet: \\floridadep\Data\DEAR\Labs\Lab3\Biology\SARTORIUS

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



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