

**FORENSIC TOXICOLOGY LABORATORY
OFFICE OF CHIEF MEDICAL EXAMINER
CITY OF NEW YORK**

PIPETTE CALIBRATION PROCEDURES

Calibration of hand held pipettes and pipetter-diluters are performed once a year and before being put into use for the first time. Uncalibrated pipettes and pipetter-diluters may not be used for analyses and must be labeled "out of service".

Materials

Analytical balance capable of readings to 4 decimal places
Weigh boats (or other suitable container)
Distilled or deionized water
Appropriate volume pipette tips
Pipette calibration data sheet for **taring** balance or **non-taring** balance

HAND HELD PIPETTE CALIBRATION PROCEDURE

Applies to but is not limited to:

Biohit pipettes
Eppendorf Repipetors
Finnpipettes
Fisher Scientific Repipetors
Oxford Repipetors
LabSafety Repipetors

1. Turn on power to balance.
2. Weigh the boat by placing on the balance pan, closing the balance doors, and releasing the toggle, freeing the balance pan to weigh.
3. If the balance has a tare function, proceed with #4, if not, go to #9.
4. Tare the weight of the boat by pressing the TARE button. The weight reading should be 0.0000 g.
5. Secure the balance pan, and pipette one volume of water into the boat.
6. Release the pan and read the weight to four decimal places.

Note: If the balance doors are closed there should be minimal effect from air currents but there may still be some variation in the fourth decimal place. Pick a consistent time after the pan is released (for instance 10 counts) to record the weight.

7. Record this first weighing and other requested data on the pipette calibration data sheet for taring balance.
8. Repeat from #4 until you have ten weight determinations.
9. If the balance does not have a tare function proceed as follows:
10. Weigh the boat and record the weight at the bottom of the pipette calibration data sheet.
11. Secure the pan and pipette one volume of water into the boat.
12. Release the pan and record the weight to four decimal places.
13. Repeat steps 11 and 12 until ten weight determinations have been made.
14. Enter data in the Excel file T:\qualitycontrol\2013\pipcal\pipcal. Choose the appropriate tab for a taring or non-taring balance.

The spreadsheet in Excel calculates the mean, standard deviation (SD) and % coefficient of variation (%CV) using the following formulas:

$$\begin{aligned}\text{Mean} &= \text{AVERAGE}(\text{cellX}:\text{cellY}) \\ \text{SD} &= \text{STDEV}(\text{cellX}:\text{cellY}) \\ \%CV &= (\text{cell} [\text{mean}]/\text{cell} [\text{SD}]) * 100\end{aligned}$$

15. Print a copy of results generated, attach the handwritten datasheet and file in pipette calibration log book.
16. Pipettes must calibrate within 5% of target volume with a CV of $\leq 1\%$.
17. Prepare a label with the following information:
 - a. Pipette Brand Name and Number
 - b. Calibration volume
 - c. Calibration date
 - d. Initials

PIPETTER-DILUTER CALIBRATION PROCEDURE

Applies to but is not limited to:

Hamilton Microlab 500 Series Pipetter-Diluter

1. Label 20 F-45 sample vials with caps.
2. Weigh each vial and cap to 0.0001 g. Record weights in column A (1-10) and D (11-20) of the validation worksheet.
3. Pipette air and diluent into vials 1-10. (Cap vials after each pipetting).
4. Pipette deionized water and diluent into vials 11-20. (Cap vials after each pipetting).
5. Weigh all vials with caps and contents to 0.0001 g. Record the weights of vials 1-10 in column B of the validation worksheet. Record the weights of vials 11-20 in column E of the validation worksheet.
6. Enter data into Excel file T:\qualitycontrol\2013\pipcal\repipcal choosing the appropriate tab. This will calculate mean difference in weight of the full and empty vials in column F. The mean value in column F must be within 5% of 0.100 g with a CV \leq 1%.
7. Prepare a label with the following information:
 - a. Hamilton Pipetter- Diluter
 - b. Calibration date
 - c. Initials
8. Print copy of results generated in Excel, attach handwritten data sheet and file in pipette calibration log book. Place a copy of the calibration on the pipetter-diluter.