

<b>KCMC Biotechnology Laboratory</b>	<b>STANDARD OPERATING PROCEDURE</b>	<b>Effective Date</b> <b>31 October 06</b>	<b>SOP-Number</b> <b>EQP.004.05</b>
		<b>Page 1 of 9</b>	<b>Date</b> <b>23 November 2007</b>
<b>Title: PIPETTE CALIBRATION</b>			
<b>Author/Date: Anne Morrissey, November 2007</b>			
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**This SOP has been read and understood by:**

Name	Date
Signatures on original copy filed in office.	
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Annual Review	
By	Date

# KCMC Biotechnology Laboratory

## STANDARD OPERATING PROCEDURE

Effective Date  
31 October 06

SOP-Number  
EQP.004.05

Page 2 of 9

Date  
23 November 2007

Title: **PIPETTE CALIBRATION**

### Document History:

Version Number	Reason for Changes	Date
EQP.004.05	Revision of Excel spreadsheet for calculating precision and accuracy.	23 November 07
	Revision of Set Volumes to correspond with pipettes in use.	
	Addition of manufacturer's Precision CV% for all test volumes.	

### Copies distributed to:

Name	Date

**Title: PIPETTE CALIBRATION**

**PURPOSE**

To assure accuracy of micropipetting devices.

**PRINCIPLE**

This is the gravimetric procedure. Set volumes of distilled water equilibrated to ambient room temperature (RT – 18-25° C) are weighed 10 times on an analytical balance and the weights entered into the Pipette Calibration Excel spreadsheet. The mean volume, Standard Deviation (SD) and Coefficient of Variation (CV) are calculated automatically. Pipetting devices that do not demonstrate acceptable accuracy or precision are removed from use, recalibrated and re-verified or removed from use.

**SCOPE**

This standard operating procedure applies to all personnel in the Biotechnology laboratory who have been trained to calibrate pipettes.

**SAFETY**

Pipettes may be contaminated with blood or human body fluids. Wear gloves and treat them as infectious during this procedure.

**MATERIALS**

Pipettor(s) to be calibrated each bearing a unique identification number/code equilibrated to RT (18-25°C).

Analytical Balance (SartoriusTE64) calibrated prior to use for pipette calibration.

Pipette tips appropriate for pipettors equilibrated to RT

Analytical balance, calibrated and capable of measuring to adequate sensitivity

Distilled water in small beaker equilibrated to RT

Pipette Calibration Record Form – See APPENDIX A (printed copy for entering data and weights)

Pipette Calibration Excel spreadsheet (see APPENDIX B for example of completed copy)

Plastic weighing boats

**FREQUENCY OF CALIBRATION**

Check the performance every 3 months and after any maintenance/adjustments. On an annual basis pipettes should be calibrated twice internally and twice by external service.

**Title: PIPETTE CALIBRATION****SELECTION OF SET VOLUMES**

Set volumes are determined by the size of the pipette being calibrated. See Table 1. If an adjustable pipette is being used for just one volume then that is the only volume that has to be calibrated and this volume should be marked on the pipette.

**Table 1: Set volumes for adjustable pipettes**

<b>Pipette model</b>	<b>Set volumes (ul)</b>
0.5 – 10 ul	10/5/0.5
10 – 100 ul	100/50/10
20 – 200 ul	200/100/20
100 – 1000 ul	1000/500/100
Repeater pipette with 2.5 ml Combitip	500/50

**WATER DENSITY**

1. The ambient air temperature will affect the density of water and the calibration calculations should be adjusted to allow for differences in water temperature.
2. Fill a beaker with the water that will be used for the calibration.
3. Allow the water to come to room temperature.
4. Take the temperature of the water with a thermometer and document the temperature.
5. Obtain the density of the water from the water density table (Table 2).

**Title: PIPETTE CALIBRATION****Table 2. Water Density Table**

<b>Temperature (° C)</b>	<b>Density</b>
15	0.9991
16	0.9989
17	0.9988
18	0.9986
19	0.9984
20	0.9982
21	0.9980
22	0.9978
23	0.9975
24	0.9973
25	0.9970
26	0.9968
27	0.9965
28	0.9962
29	0.9959
30	0.9956

**WEIGHING PROCEDURE**

*Before proceeding with the pipette calibration perform any scheduled maintenance and/or cleaning. Refer to manufacturer's instructions for individual pipettes.*

1. Prior to calibrating pipettes, the analytical balance must be checked and calibrated. Refer to EQP.006 BALANCE CALIBRATION SOP for daily balance maintenance procedures.
2. If you are calibrating an adjustable pipette. Select the appropriate volume to be weighed (see Table 1).
3. Carefully fit the pipette tip onto the tip cone.
4. Fill the tip with test water and expel to waste five times to achieve humidity equilibrium in the dead air volume.
5. Replace the tip.
6. Pre-wet the tip by filling it once with test water and expel to waste.
7. Place the empty weighing boat on the balance and tare.

**Title: PIPETTE CALIBRATION**

8. Aspirate the test water, immersing the tip only 2-3 mm below the surface of the water. Keep the pipettor vertical.
9. Withdraw the pipettor vertically and touch the tip against the inside wall of the weighing boat and expel the water. Close the door of the balance and allow the balance to equilibrate before taking the reading.
10. Determine the first weight in milligrams (mgs) and record on the Pipette Calibration Record in Box #1, Column #1 of Test Weights. Repeat the same process for the next 9 readings, pipette the water into the weighing boat by touching the tip against the inside wall of the weighing boat just above the liquid surface at an angle of 30° to 45°. Withdraw the pipettor by drawing the tip 8-10 mm along the inner wall of the weighing boat. Tare the balance between each reading.
11. Read and record the 9 weights on the Pipette Calibration Record in the appropriate boxes.
12. Enter the following information into the electronic Pipette Calibration Record Excel spreadsheet:
  - a. Date
  - b. Temperature
  - c. Density of Water
  - d. Operator name
  - e. Pipette Information
  - f. Set Volume being tested
  - g. Individual weight measurements
  - h. Acceptable CV Precision (See Table 3.)
  - i. Acceptable Accuracy % (See Table 4.)
  - j. Calculations will perform automatically for the mean, SD and CV
  - k. Indicate on the form if the accuracy and precision are acceptable
13. If the calibration is successful, label the pipette with the calibration date, calibration expiration, calibration range and the initials of the person who performed the procedure.

**CALCULATION OF ACCURACY AND PRECISION**

1. The mean volume of water will be calculated from the weight and water density and will appear in the indicated box.

**Title: PIPETTE CALIBRATION**

- The Standard Deviation (SD) and Coefficient of Variation (CV) will also be calculated by the spreadsheet and appear in the appropriate boxes.

**ACCEPTABLE RESULTS****Table 3. Imprecision CV% (manufacturer's specifications)**

Pipette Volume Range	Set Volumes	Precision CV% $\pm$
0.5 – 10 ul	10/5/0.5	0.6/1.0/4.0
10 – 100 ul	100/50/10	0.15/0.40/1.0
20 – 200 ul	200/100/20	0.15/0.30/0.80
100 – 1000 ul	1000/500/100	0.20/0.25/0.70
Repeater pipette with 2.5 ml Combitip	500/50	0.3/0.8

**Table 4. Acceptable Accuracy Deviation %**

Volume measured ( $\mu$ l)	Acceptable Deviation $\pm$
0.2 -0.4	20%
0.5-50	4%
51-1000	2%

- The coefficient of variation (CV) must be lower than the CV% specified by the manufacturer for the pipette being calibrated. Enter the appropriate CV% in to the box marked Precision (CV %) on the top of the spreadsheet. For the calibration to be acceptable the CV% calculated for the pipette must be lower than the CV% target.
- The mean of the 10 dispensed volumes must be within the acceptable limit of the set volume to assure accuracy (see Table 4). Enter the acceptable % deviation of the pipette into the appropriate box on the spreadsheet and the acceptable lower and upper limits will be calculated automatically.
- Print the form.
- Evaluate the results to see if the results for accuracy and precision are acceptable and place ticks/checks or Xs in the appropriate boxes.
- Complete the information at the bottom of the form:

**Title: PIPETTE CALIBRATION**

- a. Check the appropriate box to indicate if the results are acceptable or unacceptable for both accuracy and precision.
- b. If any results are unacceptable, record any corrective action taken (see below for suggested corrective actions).
- c. Following corrective action, calibrate the pipette again using the same form – there are two more columns for additional calibrations on each form.
- d. Sign and date the form.
- e. Submit to supervisor for review.

**CORRECTIVE ACTIONS**

1. Examine the pipette and repeat maintenance if necessary. Check that the “O” rings are seated properly and that the pipette is well greased.
2. Repeat the calibration procedure.
3. If still unacceptable, remove the pipettor from use.
4. In some cases, it may be possible to recalibrate or service the pipettor in the laboratory. Servicing might include changing a part, cleaning the pipette or other procedures. Refer to manufacturer’s instructions. Never attempt to recalibrate or service a pipette without speaking with a supervisor.
5. If still unacceptable, discard pipettor or send to the manufacturer for service.

**CALCULATIONS**Standard Deviation Formula

$$s = \sqrt{\frac{\sum(x_i - \bar{x})^2}{(n - 1)}}$$

Coefficient of Variation Formula

$$CV\% = \frac{\text{Standard deviation}}{\text{mean}} \times 100$$

Total Volume of Water

$$\text{Total Volume of Water} = \frac{\text{Weight}}{\text{Density}}$$



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		<b>Page 9 of 9</b>	<b>Date</b> <b>23 November 2007</b>
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**REFERENCES –**

**SMILE-INT SOP# cal/ver105 Version #1.0 May 27, 2005**

**APPENDIXES:**

**APPENDIX A KCMC Pipette Calibration Spreadsheet**

**APPENDIX B KCMC Pipette Calibration Spreadsheet example**