PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Title: Cleaning, Calibration and Operation of Micropipettes				
SOP No.:		**Department:**	Microbiology	
		Effective Date:		
Revision No.:	00	**Revision Date:**		
Supersede Revision No.:	Nil	**Page No.:**	1 of 5	

1.0 OBJECTIVE

To lay down procedure for the cleaning, calibration and operation of micropipettes.

2.0 SCOPE

This SOP is applicable for micropipettes used in microbiology laboratory.

3.0 RESPONSIBILITY

Prepared by - Executive Microbiology

Checked by - Assistant Manager Microbiology / QC

Approved by - Head QA, QC

4.0 PROCEDURE

4.1 Pipetting (Aspirating and Dispensing)

4.1.1 Ensure that the auto pipette has been calibrated before its use.

4.1.2 Use only clean dry and sterilized tips.

4.1.3 Adjust the volume of the auto pipette as per the requirement and fit a tip on the nozzle of the auto pipette.

4.1.4 Fix the tip to the tip holder with slight twist motion to ensure positive airtight seal.

4.1.5 Dip the pipette tip into the solution and rinse the tip once with the solution before actual Pipetting.

4.1.6 Again dip the pipette tip into the solution. The extent to which the tip should be dipped in the solution shall depend on the amount of solution to be withdrawn.

4.1.7 Aspirate the solution and leave the tip immersed in the solution for about a second after Pipetting to avoid intake of air.

4.1.8 Ensure that the solution does not adhere to the exterior of the tip. If required, wipe with a clean tissue paper.

4.1.9 Place the tip against the wall of the vessel into which the solution is to be dispensed.

4.1.10 Dispense the solution into the vessel. After dispensing the solution, eject the tip of the auto pipette.

4.2 Cleaning

4.2.1 Externally pipette can be cleaned with moist lint free duster. If required use isopropanol to moisten the cloth.

4.2.2 If the push button movement is not free, the pipette needs to be disassembled and cleaned internally.

4.2.3 For this remove tip ejector and then unscrew the connecting nut.

4.2.4 Rinse the tip holder, piston and O –ring with purified water.

4.2.5 Dry these parts thoroughly before reassembling the pipette.

4.2.6 While reassembling the pipette hand tighten the connecting nut. Never try to over tighten any connection of the pipette.

4.3 Calibration

4.3.1 Calibrate the pipette at three different volume points (Theoretical volume) that lie in between its volume range (Preferably at lowest, highest and one in between the lowest and highest volume points).

4.3.2 Calibrate the pipette at room temperature and use the density value of water at that temperature (Refer table - I for density determination).

4.3.3 Set the volume at which the pipette is to be calibrated.

4.3.4 Record the temperature of the WFI using the calibrated thermometer. Each pipette being calibrated must have 15 - 20 measurements taken of each volume, using a different pipette tip for each measurement.

4.3.5 The pipettes must use the same type of pipette tips that were used in the calibration. If a pipette tip to be used for a pipette is a type other than the type used in the calibration, the pipette must be recalibrated using the different tip. The pipette will then be calibrated for both types of tips.

4.3.6 Calculate the mean and standard deviation for each pipette and for each volume. Determine the theoretical weight of water delivered of each volume. Perform the calculations for Percent root mean bias (% RMB) and Percent coefficient variation (% CV) for each pipette and for each volume.

4.3.7 Calculations -

(a) Theoretical Wt. = (Theoretical Volume in ml) x (density of Water g / ml)

(b) $$\%\ RMB = \frac{(\text{Mean Wt. of Water}) - (\text{Theoretical Wt. of water}) \times 100}{\text{Theoretical Weight of Water}}$$

(c) $$\%\ CV = \frac{\text{Standard Deviation of the Water Weights} \times 100}{\text{Mean Weight of Water}}$$

Table - I
Water Densities in g / ml

Temperature (°C)	Density (g / ml)	Temperature (°C)	Density (g / ml)
16	0.9989430	26	0.9957837
17	0.9987749	27	0.9965732
18	0.9985956	28	0.9962335
19	0.9984052	29	0.9959448
20	0.9982041	30	0.9956473
21	0.9979925	31	0.9953410
22	0.9977705	32	0.9950262
23	0.9975385	33	0.9947030
24	0.9972965	34	0.9943715
25	0.9970449	35	0.9940319

4.4 Acceptance criteria

4.4.1 The % RMB must be not more than one percent (NMT 1%) at all volumes.

4.4.2 The % CV must be not more than one percent (NMT 1%) at all volumes.

4.4.3 Frequency of Calibration is once in a month.

4.4.4 Calibration data is to be recorded in the annexure - I.

5.0 SAFETY & PRECAUTIONS

5.1 To prevent solution from entering into the auto pipette, do not shake, turn or lay down the auto pipette horizontally.

5.2 Change in the volume adjustment should be done gently with care in order to avoid damage.

5.3 Pre rinse each new tip if to be used with liquids with high velocity (e.g. glycerol).

5.4 Dip the tip in the liquid to minimum depth required.

5.5 Discard the pipette tip if it already contains droplet of liquid.

5.6 Do not use the pipette for liquids above 70°C.

5.7 Do not allow any liquid to enter the tip holder of the pipette.

5.8 Never turn the pipette upside down.

5.9 Never lay the pipette on its side when liquid is present in the tip.

5.10 Always hold the pipette in vertical position.

5.11 Recalibrate the pipette after every internal cleanup.

6.0 REVISION HISTORY

Revision No.	Reason for Revision	Superseded from & date
00	First Issue	--------

7.0 REFERENCES

Not applicable

8.0 ABBREVIATIONS

SOP	:	Standard Operating Procedure
°C	:	Degree centigrade
%	:	Percentage
WFI	:	Water for injection
NMT	:	Not more than

9.0 ANNEXURES

Annexure - I : Calibration record of micropipette

ANNEXURE - I

CALIBRATION RECORD OF MICROPIPETTE

AutoPipette ID: _____ Auto pipette range: _____

Dateof calibration: _____ Due date of calibration: _____

Thermometer: _____ WFI Temperature_____

Balance ID : _____

Acceptance Criteria: % RMB & CV% should be NMT 1%

Sr. No.	Set volume: _____	Set volume: _____	Set volume: _____
	Observed wt.	Observed wt.	Observed wt.
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
Mean wt			
Theoretical wt.			
%RMB			
STDEV.			
%CV			

Remarks: The Pipette complies / Does not comply with laid down acceptance criteria.

Done by:
Date:

Checked by:
Date: