



STANDARD OPERATING PROCEDURE

| | |
|---|------------------------|
| Department: Microbiology | SOP No.: |
| Title: Procedure for Calibration of Auto pipette | Effective Date: |
| Supersedes: Nil | Review Date: |
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1.0 Objective

To lay down a procedure for Calibration of Autopipette.

2.0 Scope

This Standard Operating Procedure is applicable for formulation plant.

3.0 Responsibility

Executive/Officer - Microbiology : Shall be responsible to follow the procedure for Calibration of Autopipette

Head - QC/Designee : Shall be responsible for the compliance of this SOP.

4.0 Abbreviations and Definitions

SOP : Standard Operating Procedure

QC : Quality Control

σ (Sigma) : Standard Deviation

Δ : Difference in the weight of two subsequent volumes.

μL : Micro liter

g : Gram

5.0 Procedure

5.1 Calibration check of balance

5.1.1 Record the balance and weight set identification information in the appropriate blanks.

5.1.2 With the weight pan empty, tare the balance.

5.1.3 Select at least four of the data points from the table that span the expected range of use at each point.

5.1.3.1 Place the appropriate weight on the weighing pan.

5.1.3.2 Record the indicated weight in the column labeled 'Reading' in the report format as Annexure-1 or 2.

5.1.3.3 If the weight is within the desired tolerance, write in the column labeled 'Satisfactory' in the report format as Annexure-1 or 2.

5.1.3.4 If any data point falls outside of the required tolerance, perform a balance calibration using the appropriate procedure.



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5.2 Calibration of a Fixed-Volume Pipette

- 5.2.1 Place a small liquid scintillation vial or beaker capable of holding at least 20 times the pipette volume on the balance weighing pan.
- 5.2.2 Tare the balance and record the weight on line 0 under Run 1.
- 5.2.3 Carefully pipette deionized water into the vial. Record the balance reading to the nearest 0.0001 g range under the weight column for Run 1.
- 5.2.4 Repeat the point no.5.2.3 fifteen times, adding each aliquot to the last.
- 5.2.5 Calculate the difference in weights between subsequent aliquots. Record these values in the Δ weight column.
- 5.2.6 Calculate and record the average, standard deviation (σ), and twice the standard deviation (2σ) of the Δ weight values converted to μL (multiple the weight in grams by 1000).
- 5.2.7 Identify any aliquots for which the pipetted volume is greater than 2σ from the average value.
- 5.2.8 Calculate and record the pipette accuracy based on the following formula

$$\text{Accuracy (\%)} = \frac{\text{Pipet Volume} - \text{Average Value}}{\text{Pipet Volume}} \times 100$$

- 5.2.9 Calculate and record pipette precision based on the following formula:

$$\text{Precision (\%)} = \frac{\sigma}{\text{Average Volume}} \times 100$$

- 5.2.10 If any aliquot is greater than 2σ from the average volume, or if either the accuracy or precision significantly exceed the values listed in Table 1, repeat section D as Run 2.

5.3 Calibration of Adjustable-Volume Pipette

- 5.3.1 Adjustable pipettes shall be calibrated with at least three volumes. These volumes shall include the minimum and maximum volumes, as well as a mid-range or often



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used volume. (e.g., if a 500 - 2500 μl pipette is routinely used to dispense 2000 μl volumes, it must be calibrated at 500, 2000, and 2500 μl .)

5.3.2 For each volume to be calibrated:

5.3.2.1 Adjust the pipette to the target volume

5.3.2.2 Record the volume on the data sheet.

5.3.2.3 Place a small liquid scintillation vial or beaker capable of holding least 20 times the pipette volume on the balance weighting pan.

5.3.2.4 Tare the balance and record the weight on line 0 under Run 1.

5.3.2.5 Carefully pipette deionized water into the vial. Record the balance reading to the nearest 0.0001 g range under the weight column for Run 1.

5.3.2.6 Repeat the step 5.3.2.3 fifteen times, adding each aliquot to the last.

5.3.2.7 Calculate the difference in weights between subsequent aliquots. Record these values in the Δ weight column.

5.3.2.8 Calculate and record the average, standard deviation (σ), and twice the standard deviation (2σ) of the Δ weight values converted to μL (multiple the weight in grams by 1000).

5.3.2.9 Identify any aliquots for which the pipetted volume is greater than 2σ from the average value.

5.3.2.10 Calculate and record the pipette accuracy based on the following formula

$$\text{Accuracy (\%)} = \frac{\text{Pipet Volume} - \text{Average Value}}{\text{Pipet Volume}} \times 100$$

5.3.2.11 Calculate and record pipette precision based on the following formula:

$$\text{Precision (\%)} = \frac{\sigma}{\text{Average Volume}} \times 100$$



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5.3.2.12 If any aliquot is greater than 2σ from the average volume, or if either the accuracy or precision significantly exceed the values listed in Table 1, repeat section D as Run 2.

5.4 Documentation

5.4.1 Using a copy of the attached form titled “Report of Fixed-Volume Automatic Pipette Calibration” or “Report of Adjustable-Volume Automatic Pipette Calibration”, as applicable, write the date and pipette identification information in the appropriate blanks.

5.4.2 The “Calibration Due Date” shall be 3 months from the current date.

5.4.3 Maintain the completed data sheets in the same laboratory where the pipette is being used.

5.4.4 Upon completion of the calibration, Sign and date the data sheet, Complete and affix a calibration label to the micropipette.

5.4.5 For Adjustable volume pipettes, the dial reading corresponding to each volume shall also be recorded.

5.4.6 Accuracy (For adjustable-volume pipettes, the accuracy should be listed for each volume calibrated).

Table 1. Tolerances of fixed-volume Autopipettes

| Capacity μl | Accuracy % | Precision % | Capacity μl | Accuracy % | Precision % |
|---------------------------|---------------|----------------|---------------------------|---------------|----------------|
| 10 | ± 1.2 | <0.5 | 200 | ± 0.6 | <0.2 |
| 25 | ± 1.0 | <0.3 | 250 | ± 0.6 | <0.2 |
| 50 | ± 0.7 | <0.3 | 500 | ± 0.6 | <0.2 |
| 75 | ± 0.7 | <0.3 | 750 | ± 0.6 | <0.2 |
| 100 | ± 0.6 | <0.2 | 1000 | ± 0.6 | <0.2 |
| 150 | ± 0.6 | <0.2 | 2500 | ± 0.6 | <0.2 |



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Table 2. Tolerances of adjustable-volume Autopipettes

| Capacity μl | Accuracy* % | Precision* % | Capacity μl | Accuracy* % | Precision* % |
|---------------------------|------------------------|--------------------|---------------------------|------------------------|--------------------|
| 0.5-10 | ± 5.0 to ± 1.0 | < 2.8 to < 1.0 | 50-250 | ± 1.0 to ± 0.6 | < 0.3 to < 0.2 |
| 2-20 | ± 6.0 to ± 0.8 | < 5.0 to < 0.3 | 100-1000 | ± 1.6 | < 0.3 |
| 10-100 | ± 2.0 to ± 0.7 | < 0.5 to < 0.2 | 500-2500 | ± 1.0 | < 0.2 |

*When two values are listed, the first is for the minimum capacity, the second for the maximum.

6.0 Forms and Records

- 6.1 Report of Fixed-Volume Autopipette Calibration : Annexure-1
- 6.2 Report of Adjustable-Volume Autopipette Calibration : Annexure-2

7.0 Distribution

- 7.1 Master Copy : Documentation Cell (Quality Assurance)
- 7.2 Controlled Copies : Quality Control, Quality Assurance

8.0 History

| Date | Revision Number | Reason for Revision |
|------|-----------------|---------------------|
| | 00 | New SOP |



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ANNEXURE I

Report for Fixed-Volume Auto pipette Calibration

Date of Calibration : _____ Calibration Due Date: _____

Pipette Identification

Manufacturer: _____ Volume : _____ μL
Serial Number: _____ Laboratory Used In: _____

Calibration Check of Balance

| Weight | Reading (g) | Tolerance | Satisfactory |
|---------|-------------|----------------|--------------|
| 20.0 g | | ± 0.0002 g | |
| 10.0 g | | ± 0.0002 g | |
| 5.0 g | | ± 0.0002 g | |
| 2.00 g | | ± 0.0002 g | |
| 0.100 g | | ± 0.0001 g | |
| 0.010 g | | ± 0.0001 g | |

Calibration of Pipette

Balance Manufacturer : _____
Balance ID Number : _____
Laboratory : _____
Weight Set Serial Number: _____



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| Run 1 | | | Run 2 | | |
|------------------|------------|---------------------|------------------|------------|---------------------|
| S.No | Weight (g) | Δ Weight (g) | S.No. | Weight (g) | Δ Weight (g) |
| 0 | | | 0 | | |
| 1 | | | 1 | | |
| 2 | | | 2 | | |
| 3 | | | 3 | | |
| 4 | | | 4 | | |
| 5 | | | 5 | | |
| 6 | | | 6 | | |
| 7 | | | 7 | | |
| 8 | | | 8 | | |
| 9 | | | 9 | | |
| 10 | | | 10 | | |
| 11 | | | 11 | | |
| 12 | | | 12 | | |
| 13 | | | 13 | | |
| 14 | | | 14 | | |
| 15 | | | 15 | | |
| Average | | | Average | | |
| 1 σ | | | 1 σ | | |
| 2 σ | | | 2 σ | | |
| Accuracy | | | Accuracy | | |
| Precision | | | Precision | | |

Calibrated By/ Date _____

Checked By /Date _____



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ANNEXURE II

Report for Adjustable -Volume Auto pipette Calibration

Date of Calibration: _____ Next Calibration Due Date: _____

Pipette Identification

Manufacturer: _____ Volume Range : _____ μ L

Serial Number: _____ Laboratory Used In: _____

Calibration Check of Balance

| Weight | Reading (g) | Tolerance | Satisfactory |
|---------|-------------|----------------|--------------|
| 20.0 g | | ± 0.0002 g | |
| 10.0 g | | ± 0.0002 g | |
| 5.0 g | | ± 0.0002 g | |
| 2.00 g | | ± 0.0002 g | |
| 0.100 g | | ± 0.0001 g | |
| 0.010 g | | ± 0.0001 g | |

Balance Manufacturer : _____

Balance Serial Number : _____

Laboratory : _____

Weight Set Serial Number : _____

Calibration of Pipette

| Dial Setting | | | Dial Setting | | | Dial Setting: | | |
|--------------|------------|---------------------|--------------|------------|---------------------|---------------|------------|---------------------|
| S.No | Weight (g) | Δ Weight (g) | S.No | Weight (g) | Δ Weight (g) | S.No | Weight (g) | Δ Weight (g) |
| 0 | | | 0 | | | 0 | | |
| 1 | | | 1 | | | 1 | | |
| 2 | | | 2 | | | 2 | | |
| 3 | | | 3 | | | 3 | | |
| 4 | | | 4 | | | 4 | | |
| 5 | | | 5 | | | 5 | | |
| 6 | | | 6 | | | 6 | | |
| 7 | | | 7 | | | 7 | | |
| 8 | | | 8 | | | 8 | | |
| 9 | | | 9 | | | 9 | | |
| 10 | | | 10 | | | 10 | | |
| 11 | | | 11 | | | 11 | | |
| 12 | | | 12 | | | 12 | | |
| 13 | | | 13 | | | 13 | | |
| 14 | | | 14 | | | 14 | | |
| 15 | | | 15 | | | 15 | | |



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| Average | | Average | | Average | |
|------------------|--|------------------|--|------------------|--|
| σ | | σ | | σ | |
| 2σ | | 2σ | | 2σ | |
| Accuracy | | Accuracy | | Accuracy | |
| Precision | | Precision | | Precision | |

Calibrated By/ Date _____

Checked By /Date _____



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ANNEXURE III

Figure 1. Auto pipette calibration label for a fixed-volume pipette.

| | | |
|--------------------------|---|-------|
| Calibration Done By | : | _____ |
| Calibrated On | : | _____ |
| Next Calibration Due On: | | _____ |
| Volume | : | _____ |
| Precision | : | _____ |

Figure 2. Auto pipette calibration label for an adjustable-volume pipette.

| | | |
|-------------------------|---|-------|
| Calibration Done By | : | _____ |
| Calibrated On | : | _____ |
| Next Calibration Due On | : | _____ |
| Setting/Volume | : | _____ |
| Precision : | | _____ |
| Setting/Volume | : | _____ |
| Precision : | | _____ |
| Setting/Volume | : | _____ |
| Precision : | | _____ |