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The University of Arizona
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Standard Operating Procedure **SOP-BCO-L-9.0**

Title: Operation, Calibration, and Maintenance of Fixed- and Adjustable-Volume Pipette Guns

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Operation, Calibration, and Maintenance of Fixed- and Adjustable-Volume Pipette Guns

1.0 Purpose and Applicability

This standard operating procedure (SOP) describes general procedures for the operation, calibration, and maintenance of fixed- and adjustable-volume pipette guns.

2.0 Definitions

None.

3.0 References

3.1 "Standard Practices for Apparatus, Reagents, and Safety Precautions for Chemical Analysis of Metals," Standard E 50, American Society for Testing and Materials, Annual Book of ASTM Standards, Vol. 3.05, 1990.

3.2 "Standard Specification for Reagent Water," Standard D 1193, American Society for Testing and Materials, Annual Book of ASTM Standards, Vol. 11.01, 11.03, 1991.

3.3 "CRC Handbook of Chemistry and Physics," 61st Ed., p. F-11, 1980-81.

4.0 Discussion

The operation, calibration, maintenance and cleaning procedures for electronic and manual fixed- and adjustable volume pipettes is described.

5.0 Responsibilities

5.1 The user of any fixed- or adjustable-volume pipette gun is responsible for ensuring that the pipette gun has been calibrated within the past three months.

5.2 The person who calibrates the fixed- or adjustable-volume pipette gun is responsible for recording the procedure in the pipette gun log book, and marking the pipette gun with the calibration date and their initials.

6.0 Materials and Equipment

6.1 Manual, fixed-volume pipette guns

6.2 Manual, adjustable-volume pipette guns

6.3 Electronic pipette guns

6.4 Analytical balance, capable of weighing 0.1 mg

6.5 Calculator, capable of computing standard deviation

6.6 Beaker or other suitable container

6.7 ASTM Type II water (ASTM D 1193)

6.8 Thermometer to measure water at room temperature (~25°C)

7.0 Procedures

7.1 Operation

7.1.1 Chose the desired delivery volume.

7.1.1.1 For manual, adjustable-volume pipettes, set the volume adjustment mechanism to a midrange delivery volume. Lock the pipette so that the volume will not inadvertently change.

7.1.1.2 For manual, fixed-volume pipettes, locate the pipette fixed at the volume you wish to dispense.

7.1.1.3 For electronic pipettes, key in the desired delivery volume.

7.1.2 Attach the appropriately-sized, clean, disposable tip firmly to the pipette gun. Do not use disposable polypropylene tips with any solvents or solutions that dissolve or react with polypropylene.

7.1.3 Draw solution into pipette.

7.1.3.1 For manual pipettes, depress the plunger to the first stop, immerse the tip in the sample solution with the pipette oriented vertically, and slowly release the plunger to draw solution up into the tip.

7.1.3.2 For electronic pipettes, immerse the tip into the solution and activate the solution withdrawal button.

7.1.4 If an air bubble is visible in the tip, return the liquid and repeat.

7.1.5 If the liquid being pipetted is a volatile solvent (such as methanol), it should be drawn up and returned at least once before retaining a sample in order to allow the tip to fill with solvent vapor.

7.1.6 Extract the filled tip and touch it against the side of the solution container to remove any excess liquid.

7.1.7 Expel the solution.

7.1.7.1 For manual pipettes, slowly depress the plunger to the first stop, pause for about one second, then depress the plunger to the second stop. When pipetting a viscous solution, be sure to allow sufficient time for the tip to drain fully before depressing the plunger to the second stop.

7.1.7.2 For electronic pipettes, activate the solution delivery button.

7.1.8 No liquid should remain in the tip.

7.2 Calibration

7.2.1 Record the date and the ID # of the pipette gun in the pipette gun record book.

7.2.2 Fill a clean container with at least 15 times the volume of ASTM Type II water at which you wish to calibrate the pipette gun.

7.2.3 Using a thermometer, measure and record the temperature of the water.

7.2.4 Set the delivery volume and record it in the pipette gun record book.

7.2.5 Fit the pipette gun with the appropriate disposable pipette tip.

7.2.6 Pipette an aliquot of ASTM Type II water into a tared container, as described in Section 7.1. Record the weight in the pipette gun record book.

7.2.7 Discard the tip.

7.2.8 Repeat Steps 7.2.5 - 7.2.7 nine more times.

7.2.9 Calculate and record the mean weight of the ten replicates.

7.2.10 Calculate and record the true weight of the volume of water dispensed.

7.2.11 Calculate and record the percent accuracy of the ten replicates.

7.2.12 Calculate and record the percent relative standard deviation of the ten replicates.

7.2.13 If the pipette passes calibration, mark it with the date and your initials.

7.3 Maintenance

7.3.1 Disassemble the pipette gun according to manufacturer's directions.

7.3.2 Replace any cracked bushings or worn gaskets according to manufacturer's recommendations.

7.3.3 Clean any dirty parts with a soft cloth or cotton swab.

7.3.4 Apply vacuum grease or similiar lubricant according to manufacturer's recommendations.

7.3.5 Record any maintenance procedures in the pipette gun record book

7.4 Calculations

7.4.1 The mean weight of ten replicates is expressed as:

$$W_{mean} = (W_1 + W_2 + \ldots + W_{10}) / 10$$

where W_{mean} = mean weight (g) of the ten replicate weights; W_1 = weight (g) of the first replicate; W_2 = weight (g) of the second replicate; W_{10} = weight (g) of the tenth replicate.

7.4.2 The true weight of a volume of water at a given temperature is expressed as:

$W_{true} = V \times D$

where W_{true} = true weight (g); V = set delivery volume (mL); D = density (g/mL) of the water at a given temperature (see Table 1).

7.4.3 The percent accuracy of the mean is expressed as:

$A = (W_{mean} / W_{true}) \times 100$

where A = percent accuracy of the mean; W_{mean} = mean weight (g); W_{true} = true weight (g).

7.4.4 The percent relative standard deviation is expressed as:

$RSD = (SD / W_{mean}) \times 100$

where RSD = percent relative standard deviation; SD = standard deviation (g) of the ten replicate weights; W_{mean} = mean weight (g) of the ten replicate weights.

7.5 Quality Control

7.5.1 Pipettes are calibrated at least once every three months.

7.5.2 The percent accuracy of the mean must be within 99 - 101%, inclusive.

7.5.3 The percent relative standard deviation must be 2% or less.

7.5.4 If either the percent accuracy, and/or the percent relative standard deviation for any pipette does not fall within the specified limits, the pipette fails calibration.

7.5.4.1 Maintenance is performed on the pipette according to Section 7.3.

7.5.4.2 The pipette is recalibrated.

7.5.4.3 If the pipette still fails calibration, it must be marked as "failed calibration" and removed from use.

8.0 Records

8.1 Pipette gun calibration will be recorded in the pipette gun record book. See Table 2 for an example entry.

8.2 Electrical resistivity (megohms-cm, 25°C) of all Type II water stations will be recorded with daily use in the deionised water stations log books.

Table 1. Density of Water*

Temperature, °C	Density, g/mL
18	0.99862
20	0.99823
25	0.99707
30	0.99567

* Ref. 3.3.

Table 2. Example Entry of Pipette Calibration Recorded in the Pipette Log Book.

Date: 8-3-94
Pipette ID: Eppendorf fixed volume pipette - #1000-A
Volume: 1 mL
Temperature: 25°C
Density: 0.99707 g/mL
Calculated
True Weight: 1 mL x 0.99707 g/mL = 0.99707 g
W_1 (g) = 0.9998
W_2 (g) = 0.9999
W_3 (g) = 1.0002
W_4 (g) = 1.0001
W_5 (g) = 0.9987
W_6 (g) = 0.9988
W_7 (g) = 1.0005
W_8 (g) = 1.0001
W_9 (g) = 0.9972
W_{10} (g)= 0.9975
W_{mean} (g)= 0.99928
SD (g) = 0.001173598
A (%) = (0.9993 g / 0.99707 g) x 100 = 100.22%
RSD (%) = (0.001173598/0.99928) x 100 = 0.117%
Pipette passes calibration: YES
If pipette fails calibration:
Maintenance Performed:

Results of 2nd Calibration:

_____
Signature