



## 4.0 Gravimetric POVA Intermediate Check

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### 4.1 BACKGROUND

The initial calibration of piston or plunger operated volumetric apparatus (POVA) is performed by the manufacturer. Upon receipt of a newly obtained pipette or syringe and thereafter periodically, the calibration must be verified to substantiate that the volume delivered is both accurate and precise. This is accomplished by determining the mass of a volume of liquid of known density that has been delivered into a closed vessel.

### 4.2 SCOPE

The reliability of the volume delivered by POVA is dependent upon verification of calibration. This method sets forth the requirements for both intermediate checks and calibration. The intermediate check is performed to maintain confidence in calibration. This manual weighing technique is an option to evaluate the performance of each POVA. The procedure is most applicable when larger volumes ( $\geq 1\text{mL}$ ) are employed. This analytical method applies to air displacement pipettes as well as syringes attached to dilutors and dispensers. When warranted, an approved external service provider performs actual POVA calibration.

### 4.3 EQUIPMENT

- 4.3.1 Analytical Balance  
Capable of accurately weighing volumes of interest
- 4.3.2 Thermometer  
Long Solid-Stem  
Traceable to NIST Standards  
Subdivisions of  $\leq 0.5$  degree  
Capable of reading  $20^{\circ} - 28^{\circ}\text{C}$
- 4.3.3 Weighing Vessel with Lid  
Nonporous material  
Assorted sizes to accommodate volume under consideration
- 4.3.4 Timer  
Capable of accurately monitoring seconds  
Traceable to NIST Standards
- 4.3.5 Appropriate disposable pipette tips

#### 4.4 REAGENTS

- 4.4.1 Deionized/distilled water

#### 4.5 INTERMEDIATE CHECK PROCEDURE

##### 4.5.1 General

- 4.5.1.1 The requirement for a particular POVA to have periodic intermediate checks will be indicated in the applicable analytical method.
- 4.5.1.2 Each POVA should be tracked by its serial number and/or other unique identifier.
- 4.5.1.3 Intermediate checks of POVAs by an analyst or laboratory technician will be valid for four-months provided no maintenance was necessary during this period.
- 4.5.1.4 A POVA not in-use need not have a current intermediate check, however, the POVA must be checked prior to use for an application that requires a calibrated POVA.
- 4.5.1.5 An intermediate check must be performed any time a POVA is serviced.

##### 4.5.2 Initial set-up

- 4.5.2.1 The water used for the intermediate check process should be allowed to equilibrate at room temperature for at least two hours prior to the start of this procedure. Verify that the room and water temperature are the same prior to the start of this procedure.
- 4.5.2.2 Fill out identifying information on the top portion of POVA intermediate check worksheet.
- 4.5.2.3 For adjustable volume POVA, the volume of interest should be recorded.
- 4.5.2.4 Determine and record the water temperature on the logsheet at the beginning and at the end of determinations.
- 4.5.2.5 Place a volume of water in the weighing vessel, which completely covers the bottom of the container, and cap.
- 4.5.2.6 Place the weighing vessel on the balance and tare.

##### 4.5.3 First Evaporation Loss (e) Check

- 4.5.3.1 Record the initial weight of the vessel and the time of

recorded on the logsheet.

4.5.3.2 Remove the lid for approximately 20 seconds. This time should be adjusted to correspond to the approximate time interval between repetitions in 4.5.4.

4.5.3.3 Record a post-weight of the vessel and the time noted.

4.5.4 POVA Determinations

4.5.4.1 Use designated POVA, to dispense appropriate volume of temperature-equilibrated water into the weighing vessel and cap.

4.5.4.2 A minimum of ten individual repetitions ( $W_i$ ), along with their corresponding time, should be recorded.

4.5.4.3 Calculate the Mean Delivered Weight ( $\bar{W}$ ), record on logsheet.

4.5.5 Second Evaporation Loss (e) Check

4.5.5.1 Record the weight of the vessel and the time of recorded on the logsheet.

4.5.5.2 Remove the lid for approximately 20 seconds.

4.5.5.3 Record a post-weight of the vessel and the time recorded.

4.5.5.4 Calculate the mean evaporation weight, record on logsheet.

4.5.6 Mean Delivered Volume

4.5.6.1 From the *Table 1* obtain the conversion factor ( $Z$ ) for the mean water temperature. The conversion factor is based upon an air pressure of 1013 hPa.

4.5.6.2 Calculate the Mean Volume Delivered ( $V_t$ ) at the mean recorded temperature.

$$V_t = (\bar{W} + e) \cdot Z$$

4.5.7 Inaccuracy Calculation

4.5.7.1 Determine inaccuracy by calculating the percent error ( $E_t$ ) between the expected ( $V_o$ ) and calculated mean ( $V_t$ ) volume.

$$E_t = V_t - V_o / V_o \times 100$$

<i>Temperature °C</i>	<i>Conversion Factor (Z) (<math>\mu\text{L}/\text{mg}</math>)</i>
20.0	1.0029
20.5	1.0030
21.0	1.0031
21.5	1.0032
22.0	1.0033
22.5	1.0034
23.0	1.0035
23.5	1.0036
24.0	1.0038
24.5	1.0039
25.0	1.0040
25.5	1.0041
26.0	1.0043
26.5	1.0044
27.0	1.0045
27.5	1.0047
28.0	1.0048

*Table 1*

4.5.7.2 Record % error on log sheet.

4.5.8 Imprecision Calculation

4.5.8.1 Calculate the standard deviation ( $s$ ) for the replicate weights.

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

$n$  = Total number of repetitions

4.5.8.2 Record  $s$  on worksheet.

4.5.8.3 Determine the imprecision by calculating the coefficient of variation (CV%). This is also referred to as relative standard deviation (RSD).

$$\text{CV}\% = s \cdot 100 / \bar{W} + e$$

4.5.8.4 Record CV % on worksheet.

4.5.8.5 Worksheet must be centrally stored in the laboratory performing the intermediate check.

#### 4.5.9 Evaluation of Accuracy and Precision

Refer to Analytical Method 3.0 for *Acceptance Criteria*.

### 4.6 CALIBRATION

- 4.6.1 All pipettes and syringes crucial for the quality of quantitative analysis will be calibrated when analytical method quality control values and an intermediate check indicate unacceptable performance.
- 4.6.2 The calibration will be outsourced to an approved vendor/service provider.

### 4.7 REFERENCES

- 4.7.1 ASTM Method E-1154-89 (reapproved 2003), Standard Specification for Piston or Plunger Operated Volumetric Apparatus.
- 4.7.2 Curtis, R.H., *Performance Verification of Manual Action Pipets: Part I*, Am. Clin. Lab. 12(7):8-9; 1994.
- 4.7.3 Curtis, R.H., *Performance Verification of Manual Action Pipets: Part II*, Am. Clin. Lab. 12(9):16-17; 1994.
- 4.7.4 Byer, B.J., How to Use and Check Pipetting Equipment, Scientific Newsletters, Inc., 1977.
- 4.7.5 ISO 8655-6:2002, Piston-operated volumetric apparatus – Part 6: Gravimetric method for the determination of measurement error.

## Revision History

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Revision #	Issue Date	History
0	10/1997	Original Issue
1	11-27-2001	Reworked/reformatted
2	03-22-2005	Quality requirements detailed and updated
3	05-07-2007	Updated QA measures and reformatting.
4	06-29-2007	Added yearly outsourcing for calibration requirement
0	01-20-2011	Original issue as 4.0. Split from toxicology discipline analytical methods. Formerly AM 5.1.2.