4.0 Gravimetric POVA Intermediate Check

4.1 BACKGROUND

Upon receipt of a newly obtained pipette/dilutor and after maintenance, 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 (≥1mL) 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
Note: Balance may be used if it has been checked using either the toxicology or controlled substances analytical method.

4.3.2 Thermometer
Long Solid-Stem
Traceable to NIST Standards
Subdivisions of ≤0.5 degree
Capable of reading 20° - 28°C

4.3.3 Weighing Vessel with Lid
Nonporous material
Assorted sizes to accommodate volume under consideration

4.4 REAGENTS

4.4.1 Deionized/distilled water

4.5 INTERMEDIATE CHECK PROCEDURE

4.5.1 General

NOTE: If an analyst has been approved to use the ARTEL calibration system in toxicology it may be used following the tox method.

4.5.1.1 Intermediate checks of the POVA's calibration are only required before initial use or after maintenance of the instrument.

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 indefinitely.

4.5.1.4 A POVA not in-use 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.

4.5.3 POVA Determinations

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

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

4.5.3.3 Calculate the Mean Delivered Weight **(*W*),** record on logsheet.

4.5.4 Mean Delivered Volume

4.5.4.1 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.

4.5.4.2 Calculate the Mean Volume Delivered **(V_t)** at the mean recorded temperature.

$$V_t = (\overline{W}) \cdot Z$$

4.5.5 Inaccuracy Calculation

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

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

Temperature °C	*Conversion Factor (Z) (µL/mg)*
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

4.5.5.2 Record % error on log sheet.

4.5.6 Imprecision Calculation

4.5.6.1 Calculate the standard deviation (*s*) for the replicate weights.

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

n = Total number of repetitions

4.5.6.2 Record s on worksheet.

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

$$CV\% = s \cdot 100 / \overline{W}$$

4.5.6.4 Record CV % on worksheet.

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

4.5.7 Evaluation of Accuracy and Precision

4.5.7.1 Acceptance criteria are listed in the following table.

Imprecision (CV%)	*Inaccuracy*
1.0%	2.0%

4.5.7.2 When a history for an individual pipette or syringe is established, the tolerance limits should be fine-tuned and tightened accordingly. If the values obtained for a new dilutor dispenser are small, they should remain so. For instance if the initial imprecision value is 0.25%, obtaining a 1% imprecision value on the following intermediate check is a significant departure.

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.

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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

4.0 Gravimetric POVA Intermediate Check

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.
1	08/23/2011	Evaporation calculation deleted, Acceptance criteria added Intermediate check criteria changed Remained as Alcohol AM 4
2	1-16-13	Changes made to sections 4.1 and 4.5.1.1