Section Five
Quality Assurance

5.1 Verification of POVA Calibration

5.1.1 Option One: PCS 2™ Pipette Calibration System

5.1.1.1 BACKGROUND

Colorimetry measures the intensity of a color and relates it to the concentration of the solution. The relationship between concentration and intensity is obtained through determining the degree of light absorbance by a solution at a particular wavelength. The fraction of the incident light that is absorbed by a solution depends on the thickness of the sample, the concentration of the absorbing compounds in the solution, and the chemical nature of the absorbing compound. This relationship is defined by the Beer-Lambert law:

$A = \epsilon bc$

A = Absorbance

b = Internal path length (cm) of solution vial

c = Concentration of sample solution

ϵ = Molar absorptivity of sample solution

The ARTEL PCS 2™ pipette calibration system is a colorimetric method for an intermediate check of pipette dispensing accuracy and precision. The system utilizes a photometer coupled with NIST-traceable reagents to measure liquid delivery. The system is set up so that as additional solution (V_{P1}) is added to pre-mixed volume of blank solution (V_B), the absorbance change is proportional to the volume delivered by the pipette. The volume of solution pipetted (V_{P1}) is calculated as follows:

$$V_{P1} = V_B \left[A_1 / \epsilon bc - A_1 \right]$$

The volume of repetitions (V_{P2}) is determined by the following relationship:

$$V_{P2} = V_B + V_{P1} \left[A_2 - A_1 / \epsilon bc - A_2 \right]$$

5.1.1.2 SCOPE

The reliability of the volume delivered by piston or plunger operated volumetric apparatus 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. The ARTEL instrument utilizes a system which optimizes the application of the Beer-Lambert Law to provide a reliable, time efficient, pipette intermediate check that is traceable to NIST standards. An approved external service provider performs actual POVA calibration. This analytical

method applies to air displacement pipettes as well as syringes attached to dilutors and dispensers.

5.1.1.3 EQUIPMENT, SUPPLIES AND REAGENTS

5.1.1.3.1 PCS 2™ Instrument
5.1.1.3.2 Printer
5.1.1.3.3 Printer Paper
5.1.1.3.4 ARTEL Instrument Calibration Kit
5.1.1.3.5 Appropriate ARTEL Reagent Kit

5.1.1.4 INTERMEDIATE CHECK PROCEDURE

5.1.1.4.1 Refer to manufacturer's *Standard Operating Procedure for the PCS 2™ Pipette Calibration System and PCS 2™ Pipette Calibration System Procedure Guide.*

5.1.1.4.2 PCS 2™ Instrument Calibration Check

5.1.1.4.2.1 The calibration check of PCS 2™ instrument is valid for one-month providing the instrument stays in proper working order.

5.1.1.4.2.2 PCS 2™ calibration check printouts, and/or a copy thereof, are to be initialed and placed in PCS 2™ logbook. A copy is permissible due to the nature of the thermal paper printout.

5.1.1.4.2.3 The calibration kit lot number, imprecision and inaccuracy results of the instrument calibration check should be recorded on the PCS 2™ instrument calibration log sheet.

5.1.1.4.2.4 The results of the calibration check should be evaluated and a pass or fail indicated on the PCS 2™ instrument calibration log sheet.

5.1.1.4.3 PCS 2™ Intermediate Checks

5.1.1.4.3.1 The requirement for a particular pipette or syringe to have periodic intermediate checks will be indicated in the applicable analytical method. As a rule, all methods involving quantitative analysis require periodic checks.

5.1.1.4.3.2 Intermediate check of pipette or syringe calibration is valid for four-months provided no maintenance was necessary during this period.

5.1.1.4.3.3 PCS 2™ instrument printouts, or a copy thereof, are to be initialed and placed in PCS 2™ logbook. A copy is permissible due to the nature of the thermal paper printout.

5.1.1.4.3.4 A PCS 2™ intermediate check log sheet must be maintained for each pipette or syringe by serial number or other unique identifier.

5.1.1.4.3.5 The imprecision and inaccuracy results of the intermediate check must be recorded on the appropriate PCS 2™ calibration check log sheet.

5.1.1.4.3.6 The results of the calibration check should be evaluated as described in section 5.1.1.5.4 and a pass or fail indicated on the appropriate PCS 2™ calibration check log sheet.

5.1.1.4.3.7 A minimum of 10-data points is to be collected for each check of the pipette/syringe calibration.

5.1.1.4.3.8 A pipette/syringe not in-use need not be calibrated, however, it must have its calibration verified prior to use.

5.1.1.4.3.9 An intermediate calibration check must be performed after any pipette repair/maintenance.

5.1.1.4.4 Manufacturer Data Acceptance Criteria

5.1.1.4.4.1 Artel recommendations for Piston-stroke Pipette Tolerance Limits

Pipette Volume	***Inaccuracy***	***Imprecision***
2µL	5.0%	2.0%
10µL	5.0%	2.0%
20µL	5.0%	2.0%
100µL	5.0%	2.0%
200µL	5.0%	2.0%
1000µL	5.0%	2.0%

5.1.1.4.4.2 Recommendations for Eppendorf Piston-stroke Fixed Volume Pipette Tolerance Limits

Pipette Volume	*Inaccuracy*	*Imprecision*
1µL	±2.5%	≤1.8%
2µL	±2.0%	≤1.2%
10µL	±1.5%	≤0.8%
20µL	±1.0%	≤0.5%
100µL	±0.8%	≤0.3%
200µL	±0.7%	≤0.3%
1000µL	±0.6%	≤0.2%

5.1.1.4.4.3 Recommendations for Eppendorf Repeater Plus Pipette Tolerance Limits

	Inaccuracy	*Imprecision*
Combitip Plus 0.1mL (beige piston)		
2µL	±1.6%	≤3.0%
20µL	±1.0%	≤2.0%
Combitip Plus 0.2mL (blue piston)		
4µL	±1.3%	≤2.0%
40µL	±0.8%	≤1.5%
Combitip Plus 0.5mL		
10µL	±0.9%	≤1.5%
100µL	±0.8%	≤0.6%
Combitip Plus 1mL		
20µL	±0.9%	≤0.9%
200µL	±0.6%	≤0.4%
Combitip Plus 2.5mL		
50µL	±0.8%	≤0.8%
500µL	±0.5%	≤0.3%
Combitip Plus 5mL		
100µL	±0.6%	≤0.6%
1000µL	±0.5%	≤0.25%
Combitip Plus 10mL		
200µL	±0.5%	≤0.6%
2000µL	±0.5%	≤0.25%
Combitip Plus 25mL		
500µL	±0.4%	≤0.6%
5000µL	±0.3%	≤0.25%
Combitip Plus 50mL		
1000µL	±0.3%	≤0.5%
10000µL	±0.3%	≤0.25%

5.1.1.4.5 Intermediate Check Acceptance Criteria

5.1.1.4.5.1 Initially the tolerance limits recommended by ARTEL {5.1.1.4.4} should be applied.

5.1.1.4.5.2 When a history for an individual pipette or syringe is established, the tolerance limits should be fine-tuned and tightened accordingly.

5.1.1.4.5.3 Refer to ARTEL publication issue 5 (March 1999) for information regarding tolerance setting.

5.1.1.4.5.4 Refer to package insert for tolerance limits observed by manufacturer for each individual pipette.

5.1.1.5 CALIBRATION CHECKS

5.1.1.5.1 All pipettes and syringes crucial for the quality of quantitative analysis will be calibrated annually by a qualified external vendor/service provider.

5.1.1.5.2 The requirement that a calibrated pipette/syringe is to be used is indicated in the relevant analytical method.

5.1.1.6 REFERENCES

5.1.1.6.1 Standard Operating Procedure for the PCS 2™ Pipette Calibration System, Artel Document #310A2715A, April 1997,

5.1.1.6.2 PCS 2™ Pipette Calibration System Procedure Guide, Artel Document # 15A2135, Version 5.1, 03-28-1997.

5.1.1.6.3 ASTM Method E 1154-89 (reapproved 2003)**, Standard Specification for Piston or Plunger Operated Volumetric Apparatus.**

5.1.1.6.4 Segel, I.H., Spectrophotometry and Other Optical Methods. pp. 324-329. *In:* "Biochemical Calculations", Second ed., John Wiley & Sons, New York, 1976.

5.1.1.6.5 Kolthoff, I.M., Sandell, E.B., Meehan, E.J. and Bruckenstein, S., Absorption Spectrophotometry. pp. 967-970, *In:* "Quantitative Chemical Analysis", Fourth ed., Macmillan, New York, 1969.

5.1.1.6.6 Setting Tolerances for Pipette Performance, Artel lab report, Issue 5, March 1999.

5.1.1.6.7 Curtis, R.H., *Performance Verification of Manual Action Pipets: Part I,* Am. Clin. Lab. 12(7):8-9; 1994.

5.1.1.6.8 Curtis, R.H., *Performance Verification of Manual Action Pipets: Part II,* Am. Clin. Lab. 12(9):16-17; 1994.

5.1.1.6.9 Eppendorf Series 2000 Reference Fixed-Volume Pipettes Instruction Manual

5.1.1.6.10 Eppendorf Series 2000 Reference Adjustable-Volume Pipettes Instruction Manual

5.1.1.6.11 Eppendorf Repeater® Plus Pipette Instruction Manual

5.1.1.6.12 Eppendorf Repeater™ Pipette Instruction Manual

5.1.1.6.13 MLA Macro and Macro Selectable Pipette Operator's Manual

Section Five

Quality Assurance

5.1 Intermediate Check for Verification of Pipette Calibration

5.1.1 Option One: PCS 2™ Pipette Calibration System

Revision #	Issue Date	History
0	11-27-2001	Original Issue
1	02-02-2005	Quality requirements detailed and updated.
2	05-07-2007	Updated QA measures and reformatting.
3	06-29-2007	Added yearly outsourcing for calibration.