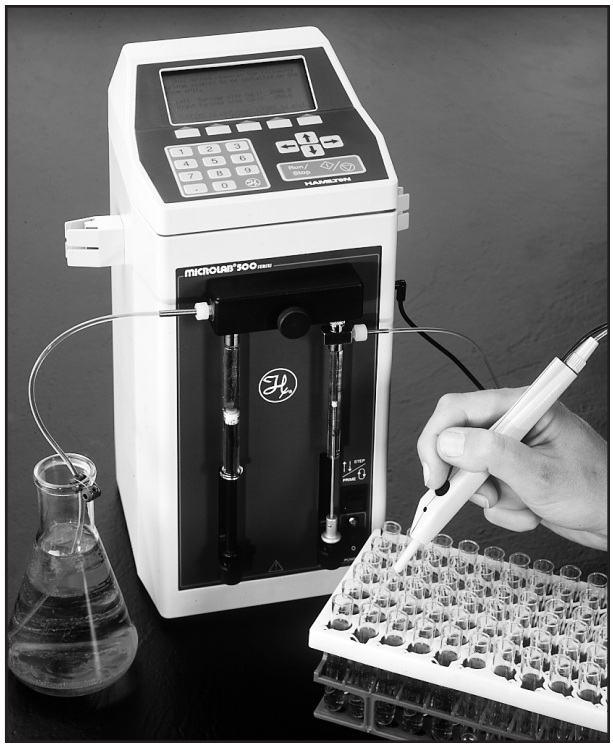
HAMILTON

THE MEASURE OF EXCELLENCE SM

Comparing the Performance of Automated Diluters with the Accuracy and Precision Standards for Class A Volumetric Glassware



Susan Mayes
Hamilton Company
4970 Energy Way
Reno, Nevada 89502 USA

Reprinted from the poster
presentation at
Pittcon '97, Atlanta
March 18, 1997

Table of Contents & Objective

Table of Contents

Objective

Automated sample preparation devices, such as the Hamilton MICROLAB® 500 (ML500), reduce preparation time, reagent volume requirements, and waste disposal costs. In addition to these benefits, laboratory managers, technicians, and auditors require that the accuracy of these instruments meets the criteria established for Class A volumetric glassware. USP methods specify the use of volumetric apparatus unless automated devices can demonstrate equivalent performance.

The following is a validation that the ML500 can be a preferred alternative to pipets, burets, and volumetric flasks.

Table 1. Accuracy specifications for Class A volumetric glassware. The applicable ASTM standards are referenced in parentheses.

	Tolerance, ±mL					
Capacity mL	**Microvolumetric Vessels (E237)**	**Burets (E287)**	**Volumetric Flasks (E288)**	**Transfer Pipets (E969)**	**Graduated Cylinders (E1272)**	**Measuring Pipets (E1293)**
0.5				0.006		
1	0.010			0.006		0.01
2	0.015			0.006		0.01
3	0.015			0.01		
4	0.020			0.01		
5	0.020		0.02	0.01	0.05	0.02
6				0.01		
7				0.01		
8				0.02		
9				0.02		
10	0.020	0.02	0.02	0.02	0.10	0.03
15				0.03		
20				0.03		
25	0.030	0.03	0.03	0.03	0.17	0.05
30				0.03		
40				0.05		
50		0.05	0.05	0.05	0.25	
100		0.10	0.08	0.08	0.50	
200			0.10			
250			0.12		1.00	
500			0.20		2.00	
1000			0.30		3.00	
2000			0.50		6.00	

Other Specifications & Analytical Comparison of Performance

Other Specifications

Table 2. Precision data from Table 4 of ASTM E542, "Standard Practice for Calibration of Laboratory Volumetric Apparatus."

Vessel	Size mL	Reproducibility mL	Reproducibility %
Transfer Pipets	1	0.002	0.2
	2	0.002	0.1
	5	0.002	0.04
	10	0.003	0.03
	15	0.005	0.03
	25	0.005	0.02
	50	0.007	0.014
	100	0.010	0.01
Flasks	10	0.005	0.05
	25	0.005	0.02
	50	0.007	0.014
	100	0.011	0.011
Burets	10	0.003	0.03
	25	0.005	0.02
	50	0.007	0.014
	100	0.012	0.012

Table 3. Accuracy and Precision data for the MICROLAB 500. The performance of the ML500 is specified by percent error at various percents of stroke, using a 1 mL syringe. Precision is represented as the coefficient of variation.

Percent of Stroke	Accuracy within ±%	Precision %
1-5	3.0	1.5
5-30	1.2	0.5
30-100	1.0	0.2

Analytical Comparison of Performance

If the specifications for Class A glassware and the MICROLAB 500 are compared at 1 mL, transfer pipets are slightly better.

Product	Tolerance
Transfer Pipet	± 6 µL
MICROLAB 500	± 10 µL
Microvolumetric Flask	± 10 µL
Measuring Pipet	± 10 µL
Buret	± 20 µL
Graduated Cylinder	± 50 µL

If the tolerance specifications are compared at the lowest volume specified, the ML500 is much better than Class A glassware.

Product	Tolerance
MICROLAB 500, 10 µL (1 mL syringe)	± 0.3 µL
Transfer Pipet, 0.5 mL	± 6 µL
Microvolumetric Flask, 1 mL	± 10 µL
Measuring Pipet, 0.1 mL (1 mL total vol.)	± 10 µL
Volumetric Flask, 5 mL	± 20 µL
Buret, 50 µL (10 mL total volume)	± 20 µL
Cylinder, 0.1 mL (5 mL total volume)	± 50 µL

Calibration & NIST Traceability

Each MICROLAB 500 is tested before leaving the Hamilton facilities. This evaluation involves a gravimetric calibration of each syringe drive at three volumes. One milliliter syringes are installed, and 10-sample tests are run at 10 µL, 50 µL, and 300 µL dispense volumes, using deionized water.

The ML500 used in this study was calibrated at these volumes and many others. Please refer to the Experimental portion of this presentation.

A calibration procedure, describing the details of testing these instruments gravimetrically, is found on page 10 of this poster reprint. The procedure is based on the method found in ASTM E1154, "Standard Specification for Piston or Plunger Operated Volumetric Apparatus."

The ML500 is calibrated via an unbroken chain of calibrations traceable to the National Institute of Standards and Technology (NIST). The links in the chain of traceability and the associated uncertainties are illustrated in Table 4.

Table 4. NIST traceability of the ML500.

Parameter	Step	Description	Uncertainty ±
Temperature	1	NIST calibration	0.00006 K
	2	Vendor standard	0.005 K
	3	Vendor probe	0.05 K
	4	Hamilton probe	0.05 K
	5	Fluid temperature	
Mass	1	NIST calibration	0.00000281 g
	2	Vendor standard	0.000005 g
	3	Hamilton standard	0.000007 g
	4	Hamilton balance	0.000005 g
	5	Fluid mass	

Experimental

Summary

HPLC of acetaminophen will be the vehicle for comparing the MICROLAB 500 with Class A pipets, burets, and volumetric flasks. Five concentrations of acetaminophen will be prepared using four methods: Large-volume volumetric ware, small-volume volumetric ware, the ML500 with large-volume syringes installed, and the ML500 with small-volume syringes installed. The calibration curves resulting from replicate injections of each sample concentration prepared with each method will be generated and compared. In addition, the quantity of methanol and the amount of time required to prepare the samples with each method will be monitored.

Equipment

MICROLAB 530B Diluter/Dispenser
Hamilton Syringes, 50 µL, 500 µL, 1.0 mL, 10.0 mL
Class A Pipets, Pyrex, 1 mL, 4 mL, 10 ml
Class A Buret, Pyrex, 50 mL
Class A Volumetric Flasks, 25 mL, 100 mL, 200 mL, 500 mL, 1000 mL, 2000 mL
HPLC System:
- Metering Pump, LDC/Milton Roy, Constametric IIIInjector, Rheodyne, with 10 µL Sample Loop
- Absorbance Detector, Kratos Analytical Spectroflow 757
- Integrator, Hewlett-Packard 3396 Series II
- Column, Hamilton PRP-1, 5 µm, 150x4.1 mm

Sartorius Balance, Model R160P,
- Sensitivity ±0.01 mg

Sartorius Balance, Model MC5,
- Sensitivity ±0.001 mg

Temperature Gage, Solomat MPM with platinum Pt100 probe
Weighing Vessels, 50 mL plastic beaker with parafilm cover, 300 µL microcup with lid

Chemicals

Methanol, J.T.Baker, "Baker Analyzed" HPLC Solvent
Deionized water, Milli-Q Reagent Water System
Acetaminophen, Sigma Reference Standard, Product number A-3035

Calibration

Each pipet was gravimetrically evaluated to assure Class A accuracy. The MICROLAB 500 was evaluated at the experimental volumes, both in the dispenser mode and in the diluter mode. The results are shown in Table 5.

Experimental

Table 5. Calibration results for the ML500. Each calibration at each volume involved 10 samples. Accuracy is reported as percent error (inaccuracy); precision is reported as the coefficient of variation (CV) in percent. For comparison, the specifications for volumetric pipets (per E969) are listed, where applicable, in terms of percent error (calculated from the published tolerance).

Syringe Volume	Dispensed Volume µL	Dilution Ratio	Error %	Precision %	Pipet Error %
50 µL	5	n/a	-0.45	0.51	
	10	n/a	-0.849	0.250	
	20	n/a	-0.643	0.170	
	40	n/a	0.003	0.137	
	50	n/a	-0.372	0.093	
1.0 mL	10	n/a	0.078	1.034	
	50	n/a	-0.151	0.304	
	300	n/a	0.167	0.143	
	950	n/a	0.069	0.011	
	1000	n/a	0.081	0.018	0.6
50 µL and 1.0 mL	1000	1:199	0.059	0.041	
	1000	1:99	0.049	0.008	
	1000	1:49	0.058	0.012	
	1000	1:24	0.056	0.021	
	1000	1:19	0.048	0.058	
500 µL	50	n/a	-0.020	0.431	
	100	n/a	0.014	0.287	
	200	n/a	0.174	0.059	
	400	n/a	0.071	0.065	
	500	n/a	0.044	0.047	1.2
10.0 mL	9500	n/a	0.247	0.019	
	10000	n/a	0.258	0.006	0.20
500 µL and 10 mL	10000	1:199	-0.284	0.013	
	10000	1:99	-0.289	0.013	
	10000	1:49	-0.301	0.009	
	10000	1:24	-0.308	0.011	
	10000	1:19	-0.318	0.009	

Sample Preparation

First, an acetaminophen concentrate of 1mg/mL in 3:1 water:methanol was prepared. From that, five dilutions were prepared, also with 3:1 water:methanol as the diluent, using each sample preparation method. Table 6 is a summary of the equipment used.

Table 6. Summary of syringes and volumetric glassware used to prepare dilutions.

Sample Concentration	ML500, Small	ML500, Large	Class A, Small	Class A, Large
0.005 mg/mL	5 µL of 50 µL syringe 9950 µL of 1 mL syringe	50 µL of 500 µL syringe 9.95 mL of 10 mL syringe	1 mL pipet 200 mL flask	10 mL pipet 2000 mL flask
0.01 mg/mL	10 µL of 50 µL syringe 990 µL of 1 mL syringe	100 µL of 500 µL syringe 9.90 mL of 10 mL syringe	1 mL pipet 100 mL flask	10 mL pipet 1000 mL flask
0.02 mg/mL	20 µL of 50 µL syringe 980 µL of 1 mL syringe	200 µL of 500 µL syringe 9.80 mL of 10 mL syringe	4 mL pipet 200 mL flask	10 mL pipet 500 mL flask
0.04 mg/mL	40 µL of 50 µL syringe 960 µL of 1 mL syringe	400 µL of 500 µL syringe 9.60 mL of 10 mL syringe	1 mL pipet 25 mL flask	4 mL pipet 100 mL flask
0.05 mg/mL	50 µL of 50 µL syringe 950 µL of 1 mL syringe	500 µL of 500 µL syringe 9.50 mL of 10 mL syringe	10 mL pipet 200 mL flask	25 mL of a 50 mL buret 500 mL flask

Experimental Results & Calculating Return on Investment

Chromatographic Conditions

Six injections of each of the 20 samples were chromatographed, in a manner similar to the assay described in the USP monograph. Operating conditions: Flow rate, 2 mL/min; temperature, ambient; injection volume, 10 µL; mobile phase, 3:1 deionized water:methanol; detection, 243 nm.

Results

The results are summarized in Figure 1 and Table 7.

Table 7. Comparing cost, time, and statistical regression results. The cost of methanol was based on $28 per 4 L bottle, and the cost of waste disposal was based on $520 per 55 gallon drum.

Method	**ML500, Small**	**ML500, Large**	**Class A, Small**	**Class A, Large**
Volume Methanol Used	1.25mL	12.5mL	186 mL	1040 mL
Cost of Methanol Used	$0.01	$0.09	$1.30	$7.28
Volume Waste Generated	5 mL	50 mL	742 mL	4259 mL
Cost of Waste Generated	$0.01	$0.13	$1.85	$10.40
Sample Preparation Time	25 min	25 min	75 min	75 min
Clean Up Time	5 min	5 min	15 min	25 min
Total Cost	**$0.02**	**$0.22**	**$3.15**	**$17.68**
Total Time	**30 min**	**30 min**	**90 min**	**100 min**
Best Fit Line Data				
y-intercept	456	1644	-3285	-2894
slope	11.76×10^6	12.27×10^6	12.44×10^6	12.46×10^6
R^2	0.996568	0.999459	0.999115	0.997672

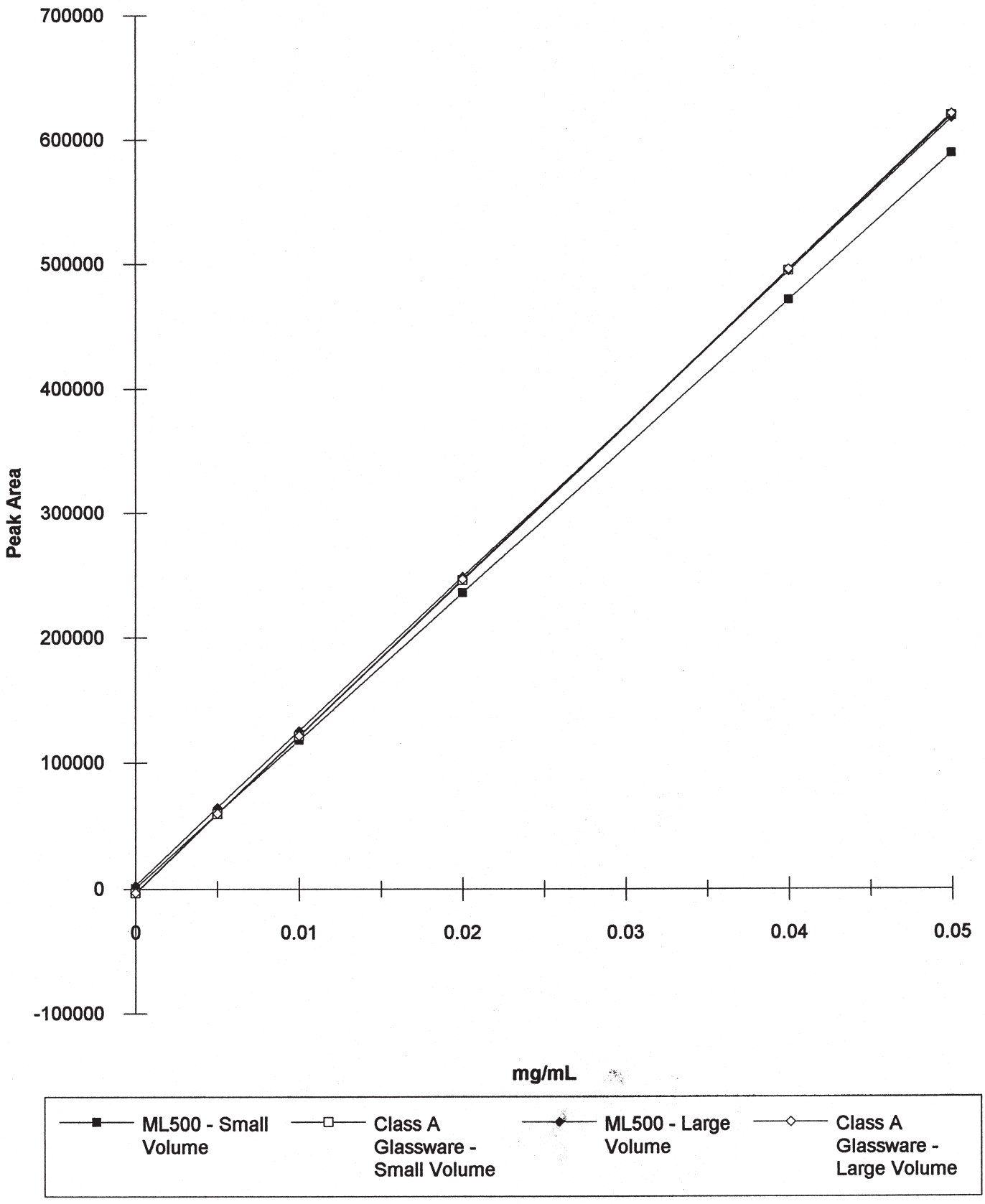
Calculating Return on Investment

The price of the ML530B is $3,500. Calculating the differences, large and small, as obtained in Table 7, and assuming a technician's hourly wage of $10, the return on investment (ROI) is between 1.5 and 3.4 weeks.

	Class A, Large vs. ML500, Small	**Class A, Small vs. ML500, Large**
Solvent Costs:	$17.68 - 0.02 = $17.66	$3.15 - 0.22 = $2.93
Labor Costs:	$10 x (100 - 30)/60 = $11.67	$10 x (90 - 30)/60 = $10.00
Cost per Set:	$17.66 + $11.67 = $29.33	$2.93 + $10.00 = $12.93
# Sets:	$3500 / $29.33 = 119	$3500 / $12.93 = 271
ROI:	119 x 0.5 hr = 60 hours	271 x 0.5 hr = 136 hours

Results

Figure 1. Best Fit of Data For Each Sample Preparation Method



Discussion & Validation Methods

Discussion

The MICROLAB 500 demonstrated superior performance in terms of cost savings (by a factor of 884) and time reduction (by a factor of 3.3).

From Figure 1, it is apparent that the ML500 methods are not significantly different from the volumetric glassware methods. The best-fit line for the ML500 small volume method falls below the others because of the relative inaccuracies of the 50 µL and 1 mL syringes used. From Table 5, the 50 µL (sample) side generally under-dispensed (negative error), and the 1mL (diluent) side generally over-dispensed. Both syringes performed within specification; however, the result was dilutions that had lower-than-nominal concentrations.

The precision of the two general methods is comparable; however, the ML500 slightly out-performed the glassware. Judging by the best-fit y-intercepts, the ML500 lines gave values that were closer to zero.

Validating Sample Preparation Methods

The experiment presented in this poster is just one example of validating an automated sample preparation method.

The primary validation protocol is to gravimetrically compare the MICROLAB 500 with the volumetric glassware that is ordinarily used in a particular procedure. Determine the accuracy and precision of both, using dispense volumes that match those that would actually be used in preparing samples.
If the performance of the ML500 meets or exceeds that of the glassware, then the ML500 is deemed a suitable equivalent.

If the ML500 does not at first meet the defined specifications, assure that the factors affecting performance (next section) have been addressed. In addition, the accuracy of the individual syringes used on the ML500 contribute significantly to the accuracy of the instrument. Different syringes may provide better performance.

A supplementary method of validating an automated sample preparation method is to actually prepare the samples with both the glassware and the instrument, and compare analytical results, as was done in the experimental section of this presentation. Each application must be evaluated for suitability, on a case by case basis.

Factors Affecting Performance, Conclusions & References

Factors Affecting Instrument Performance

- Choose the appropriate parts

  Syringes must be chosen based on the sample sizes required. For best performance, dispensed volumes should be between 10% and 80% of total syringe volumes.

  Tubing gauge must be of the correct size. For small volumes, use the smaller gauge (18). For relatively highly viscous fluids, use the large gauge (12). Assure that the outlet tubing is tapered.

  Hand probes are available for various applications.

- Installation, operation, cleaning, and maintenance

  Install syringes according to the manual's instructions.

  Operation parameters are dependant upon the type of fluids used. Liquids with low vapor pressures will require slower fill/aspiration speeds in order to avoid degassing the fluid while in the fluid path. Liquids with high viscosity may require lower speeds in order to avoid overloading the syringe drives.

  Bubbles in the fluid path may affect accuracy, especially if they break loose and are dispensed. Cleaning the fluid path may prevent bubble formation.

  Syringe plunger tips can be easily damaged if not handled properly. Pre-wet the tip before installation into the barrel. Avoid scratching or marring the plunger tip; replace damaged plunger assemblies with new ones. Leaks can result from a damaged tip, thereby affecting accuracy and precision.

  Rinsing the fluid path, especially between applications with different fluids and after a work shift where salt solutions are used, will prevent damage to the syringe plunger tips and the valves. Halogenated solvents, if left in the fluid path, may reduce the life of the adhesive between the glass syringe barrel and the TLL fittings; a thorough rinsing of these fluids after use is required.

Conclusions

In comparison with volumetric glassware, the MICROLAB 500 provides extraordinary cost savings and sample preparation time reductions. Its accuracy tolerances far exceed those of volumetric flasks, graduated cylinders, measuring pipets, and burets. Only transfer pipets can shower better accuracies. The precision of the ML500 meets or exceeds that of Class A glassware, especially when operator imprecision is considered; that is, the automated nature of the ML500 eliminates operator-to-operator inconsistencies.

Although the ML500 cannot claim better accuracy in all comparisons with Class A apparatus, its benefits weigh heavily in favor of automating small-volume sample preparation. This is especially true, since validations of individual preparation methods are relatively simple.

References

1. The United States Pharmacopeia (USP 23), The National Formulary (NF 18), United States Pharmacopeial Convention, Inc., Rockville, MD, 1995.

2. 1996 Annual Book of ASTM Standards, Section 14, General Methods and Instrumentation, Volume 14.02, American Society for Testing and Materials, West Conshohocken, PA, 1996.

3. Official Methods of Analysis of the Association of Official Analytical Chemists, Fifteenth edition, AOAC, Arlington, VA, 1990.

4. Hamilton MICROLAB 500B/C Series User's Manual, Revision C.

Instrument Calibration

Diluters and dispensers, such as the MICROLAB 500, can be periodically calibrated using the following procedure, which is a gravimetric test based on a mix of Hamilton's QC method and the method outlined in ASTM E1154, "Standard Specification for Piston or Plunger Operated Volumetric Apparatus." The procedure is rather generic, allowing the instrument user to set his/her own specifications for accuracy and precision. (Published specifications for new instruments are found in the User's Manual, and original test results are shown on the Performance Test Report(s) shipped with the instrument.) The user can specify desired test volumes, drive speed, and other conditions, according to the particular applications and requirements.

I. Summary

The general procedure is based on determining the weighing results of water samples delivered by the instrument. Volume dispensed is calculated based on the density of water at specific temperatures.

II. Limitations

This method is not recommended for volumes below 1 µL, and certain procedural modifications are required for volumes of 25µL and less. There is no upper volume limit.

III. Equipment, Materials, Environment

A. Laboratory balances required for the test method should meet or exceed the following performance specifications, be calibrated regularly with the appropriate traceable weights, and be regular ly maintained.

Test volume, µL	Balance sensitivity, mg
1-10	0.001
10-100	0.01
100+	0.1

B. Use a balance table, or suitable equivalent to minimize vibration. Cover its working surface directly in front of the balance with a dark, smooth, nonglare material. Keep the balance area reasonably free of draft currents and the ambient area free of excessive dust.

C. Use a calibrated thermometer.

D. Use a weighing vessel that has a total volume about 10 to 50 times the test volume. If possible, also use a cover that fits over the outside of the vessel top (don't allow the cover to come into contact with the test liquid). The vessel should be plastic, glass, metal, or some other nonporous material. The cross-sectional area of the opening should be as small as possible for evaporation control.

E. Handle the vessel with forceps or tweezers.

F. Use deionized water.

IV. Procedure

A. Introduction: Deliver a total of n samples into a weighing vessel, and weigh each sample after delivery. Replicate all motions and time intervals in each sampling cycle as precisely as possible. Keep the distance between the balance and the diluter/dispenser to a minimum.

B. Preparation: Select the analytical equipment and materials. Prepare the instrument to be evaluated by installing the desired syringe(s), tubing, hand probe, valve or valve assembly. Program the instrument in order set the desired dispense volumes and syringe drive speeds. Ensure that the room, equipment, and materials, including the prepared water, are thermally equilibrated. Ensure that electronic balances have had sufficient warm-up time to stabilize.

C. Place a small amount of water in the weighing vessel (between 2 and 30 sample amounts).

D. Place the instrument's inlet tubing into a water reservoir. Prime the instrument. Perform one aspirate/dispense cycle and discard the effluent. (When testing the sample side of a diluter, use the probe to aspirate and dispense the water.) Change the drive speeds if undue splashing of the dispense occurs.

E. Open door of balance chamber, place weighing vessel on balance pan, and close door of balance chamber.

F. Tare the balance. Aspirate one sample. Retrieve weighing vessel from the balance chamber, deliver complete sample, and return the vessel to the balance pan, closing the door to the chamber. Observe and record balance readout. (In some instances, it may be possible and more appropriate to dispense into the vessel without removing it from the balance.)

G. Repeat step F until 10 samples have been weighed. Note: Perform the weighing cycles as quickly as possible, but without compromising the integrity of the liquid delivery or the precision of the technique of the operator.

H. Measure and record the water temperature.

V. Procedure Modifications

Calibration (continued)

A. For volumes of 25 µL and less, follow these guidelines:

   1. Use a very small vessel, such as a microwell cup having a total volume of about 300 µL. Avoid handling the vessel by hand, as finger oils will provide a source of error. Assure a cap for the vessel is used as well.

   2. Dispense the aliquot onto the inside wall of the vessel, and not directly into the mass of water.

   3. Determine and use an evaporation coefficient. Without dispensing any sample, replicate the weighing routine. Repeat to obtain 10 values, each representing the amount evaporated from the vessel during each cycle. Add the average of these readings to each sample weighing. See the next section, Calculations.

B. To further optimize the procedure (in addition to the above small-volume guidelines):

   1. Use degassed water

   2. Use the density of water from the CRC Handbook table, based on the temperature read to the nearest 0.1 ˚C. (The table in this procedure only lists the densities based on temperatures read to the nearest 1 ˚C.)

   3. Assure that the relative humidity of the testing environment is 45-75%.

   4. Assure that the temperature of the testing environment and equipment remains constant to ±0.5 ˚C during the course of the test, and that no direct sunlight enters the testing area.

C. Here are some guidelines for various sample sizes:

   1. For validation of a new dispense/dilution method, use a sample size of 30 instead of 10.

   2. For quick performance checks, such as at a monthly preventative maintenance interval or when tubing or valves are replaced, use a sample size of 4.

   3. When a new syringe is installed onto the instrument for the first time, use the proscribed sample size of 10.

D. For a test liquid other than water, use that liquid's density in the calculations. Most liquids are not as well specified at various temperatures as water. If the density of the non-water liquid is only published for one specified temperature, realize that significant error may result if the test is done at a temperature different from that which the density is reported.

E. Gravimetric testing of dilutions of two different and interdependent test liquids is beyond the scope of this procedure.

Calibration Calculations

VI. Calculations

A. If an evaporation coefficient (C_{evap}) was determined, correct each mass reading (m_i):
$m_{corr} = m_i + C_{evap}$

B. Calculate the volume of each dispense (V_i) by dividing each (corrected) mass value by the density of water at the measured temperature. Refer to the table below for density values.

Density of Water at Various Temperatures.
Taken from CRC Handbook of Chemistry & Physics, 77th edition, 1996-97, page 6-10.

˚C	g/cc	˚C	g/cc
17	0.9987769	24	0.9972994
18	0.9985976	25	0.9970480
19	0.9984073	26	0.9967870
20	0.9982063	27	0.9965166
21	0.9979948	28	0.9962371
22	0.9977730	29	0.9959486
23	0.9975412	30	0.9956511

C. Single dispense (in)accuracies can be calculated from the volume dispensed (V_i) and the expected volume (V_o):
Accuracy (%) = 100 x (V_i - V_o) / V_o

D. Calculate the average dispensed volume from the individual dispensed volumes, V_i (where i is 1 to n, in this case 10):
$V_{avg} = (V_1 + V_2 + ... + V_{10}) / 10$

E. Calculate the instrument accuracy:
Accuracy (%) = 100 x (V_{avg} - V_o) / V_o

F. Calculate the standard deviation (SDEV) of the calculated volumes:
$SDEV = \{ [\sum(V_i - V_{avg})^2] / (n-1) \}^{1/2}$

G. Determine the coefficient of variation (precision):
CV (%) = 100 x SDEV / V_{avg}

HAMILTON
THE MEASURE OF EXCELLENCE℠

Hamilton Company
4970 Energy Way
Reno, Nevada 89502 USA
Toll-Free 800-648-5950
Fax +1-702-856-7259
Telephone +1-702-858-3000

Hamilton Bonaduz AG
P.O. Box 26
CH-7402
Bonaduz/Switzerland
Fax +41-81-641-2563
Telephone +41-81-641-0101

ISO 9001 CERTIFIED

Document No. L10041 (Rev A)