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# Gravimetric & Spectrophotometric Errors Impact on Pipette Calibration Certainty<sup>[1]</sup>

John P. Clark & A. Harper Shull  
Westinghouse Savannah River Company

The calibration of pipettes requires a high degree of skill and knowledge of the sources of error that can obscure the true volume of liquid delivered from a pipette. Much has been written concerning the errors associated with using air displacement pipettes. The same errors that contribute to the uncertainty of volumes of liquid delivered by pipettes operated by laboratory personnel must be identified and managed by pipette calibrators. Unless the variables are tightly controlled, it is difficult to determine if measured volumes of liquid from the pipette being calibrated are outside of accuracy limits or due to systematic errors in the calibration system.

The preparation of uncertainty budgets for the calibration process provides estimates of uncertainty associated with the values generated by the measurement system. The uncertainty estimates quantify the quality or accuracy of the calibrations. Error budgets are presented for both calibration systems. Details are provided on developing the gravimetric error budget.

## Introduction

Air displacement pipettes are used to make most of the volume measurements in chemical, environmental, medical, pharmaceutical and other laboratories. Calibration or verification is required to assure they are capable of accurate and precise measurements. National, international and manufacturer's procedures are used in testing pipettes. Currently, gravimetric and photometric methods are the most common methods used.

Much has been written concerning the errors associated with using air displacement pipettes.[2] The same errors that contribute to the uncertainty of volumes of liquid delivered by pipettes operated by laboratory personnel must be identified and managed by pipette calibrators. Unless the variables are tightly controlled, it is difficult to determine if measured volumes of liquid from the pipette being calibrated are outside of accuracy limits or due to systematic errors in the calibration system.

Many calibration organizations are seeking national accreditation to ISO/IEC 17025 General Requirement for the Competence of Testing and Calibration Laboratories.[3] In it, Section 5.4.6 "Estimation of Uncertainty of Measurement," provides the requirements for having and applying procedures for estimating the uncertainty of calibration measurements. It further specifies in section 5.4.6.3, "When estimating the uncertainty of measurement, all uncertainty components which are of importance in the given situation shall be taken into account using appropriate methods of analysis." Pipette calibration sources of uncertainty include the measurement system, the operator, environment and the pipettes. This paper will look at the sources for both the gravimetric and

spectrophotometric pipette calibration methods.

A useful resource for those developing uncertainty estimates is the ISO technical report ISO/TR 20461 "Determination of uncertainty for volume measurements made using the gravimetric method." [4] It covers:

- Modeling the measurements,
- Standard uncertainty of a measurement according to the GUM [5] requirements,
- Sensitivity coefficients to normalize each variable,
- Standard uncertainty associated with the volume delivered by the pipette,
- Standard uncertainties of measurement,
- Expanded uncertainties of measurement
- Example for determining the uncertainty of the measurement

In addition to providing uncertainty estimates, traceability to national standards must be demonstrated. This means the uncertainty of the calibration standards and instruments used in performing tests must be related to a national measurement system and included in the calculation of the measurement system uncertainty in order to achieve traceability.

Balance manufacturers publish performance specifications for repeatability, linearity, hysteresis, corner loading, etc., but do not provide uncertainty estimates for measurements made on their products. One explanation for this could be the lack of knowledge of the contributions of uncertainty from the balance user's environment, material being weighed, operator technique, etc., to the uncertainty of measurements. Therefore, it is incumbent upon the user to develop estimates of uncertainty for the



Figure 1. Gravimetric Pipette Calibration System.

gravimetric measurements used to calibrate or verify pipette accuracy and precision.

This is not the case for the Artel PCS®3 Pipette Calibration System, which is very specific for pipette calibrations. The manufacturer has a very comprehensive quality assurance program and knowledgeable scientists that have invested the time and effort developing detailed measurement uncertainty estimates. This allows the users of their systems to evaluate the adequacy of the photometric measurements against the accuracy tolerances of volumes they are calibrating. Their system is used exclusively for pipette calibrations while electronic balances can be used for many applications in the typical laboratory. Studies were done to determine the accuracy and precision of various adjustable pipettors at various settings. Examples of uncertainty estimates for both measurement systems are given below.

## Pipette Calibration Systems

A new 5-place analytical balance with a pipette calibration kit (Fig.1) and an Artel PCS®3 Pipette Calibration System (Fig. 2) were studied to determine their performance capabilities in calibrating pipettes. Several tests were conducted to evaluate the sources of uncertainty associated with both pipette calibration systems and the accuracy and precision of each. Comparisons were made between the two systems.

At the time of writing, the new ISO standard has not been published. The draft copy, ISO/DIS 8665-6 "Piston-operated volumetric apparatus - Part 6 Gravimetric test methods," states in the scope, "This standard specifies the gravimetric testing of errors of measurement of piston-operated volumetric apparatus." The emphasis is testing for errors which are measures of inaccuracy and imprecision. "These gravimetric test methods are the reference test methods which shall be used as conformity tests or type tests for declaration and certification of

conformity." Parts 1 through 6 of ISO 8655 are scheduled to become official in mid 2002. Work is underway on Part 7 "Non-gravimetric methods for the determination of measurement error," and it should become official in late 2003. This part will cover both the photometric and titrimetric test methods.

## Gravimetric Calibrations

The gravimetric pipette calibration uncertainty estimates are listed in a table that is often called an error budget and are based on the following model.

- The pipette volume is measured using the following model  $V_{20} = m \cdot Z \cdot Y$   
 $V_{20}$  = volume at 20° C  
 $m = m_2 - m_1 + m_E$  (gross weight minus the tare weight plus the wt of evaporation)  
 $Z$  = air buoyancy correction (density of the air at the time of measurement) and density of the water  
 $Y$  = coefficient of expansion for the pipette and fluid being dispensed.
- Each of these components has random or systematic errors that obscure the true value of measurements.
- The GUM requires the uncertainty sources be standardized, so their variances (squared standard deviations) can be combined by adding and taking the square root to estimate of the standard uncertainty.

The total measurement uncertainty of the pipette volumes involves multiplying the standard uncertainty by a k value to quantify the level of confidence for the total



Figure 2. Artel PCS®3 Pipette Calibration.

Pipet Calibration Uncertainty Budgets							
Pipette: L20, 20 $\mu$				20		20 $\mu$	
Balance UMT (7 places)							
Parameter	Interval	Distribution	(u) Standard	(c) Sensitivity	units	units	
	+/-	units	1.7320506	Uncertain	Coefficient	c*u (x)	
<b>Balance</b>	Uncertainty	5.68 ug	Normal	2.84 ug	1 nl/ug	2.84 nl	
	Linearity	1 ug	rectangular	0.58 ug	1 nl/ug	0.58 nl	
1st Value	Reproducibility	0.25 ug	rectangular	0.14 ug	1 nl/ug	0.14 nl	
2nd Value	Reproducibility	0.25 ug	rectangular	0.14 ug	1 nl/ug	0.14 nl	
1st Value	Resolution	0.1 ug	rectangular	0.06 ug	1 nl/ug	0.06 nl	
2nd Value	Resolution	0.1 ug	rectangular	0.06 ug	1 nl/ug	0.06 nl	
	Temp. Drift	1.0 ug	rectangular	0.58 ug	1 nl/k	0.58 nl	
	Correction for Evaporation	5 ug	rectangular	2.89 ug	1 nl/ug	2.89 nl	
<b>Water</b>	Temperature	0.2	rectangular	0.12 K	4 nl	0.46 nl	
<b>Air</b>	Temperature	1 K	rectangula	0.58 K	0.09 nl/K	0.05 nl	
	Pressure	27 hPa	rectangula	15.59 hPa	0.024 nl/hPa	0.37 nl	
	Relative Humid	10 %	rectangula	5.77 %	0.002 nl/%	0.01 nl	
<b>Pipette</b>							
	Cubic Expansion Coefficie	9.00E-05 K-1	rectangular	0.0001	-40000 nl*K	-2.08 nl	
	Temperature	2 K-1	rectangular	1.15	0.02 nl/K	0.02 nl	
<b>Standard Uncertainty Associated with the Volume <math>V_{20}</math> Measured with the gravimetric measuring system</b>							<b>4.18 nl</b>
<b>Precision on the calibration</b>							<b>15.97 nl</b>
<b>Combined Standard Uncertainty of the Calibration</b>							<b>16.51 nl</b>
<b>Converted to microliters =</b>							<b>0.017 <math>\mu</math></b>
<b>Expanded Uncertainty @ k = 2</b>							<b>0.033 <math>\mu</math></b>
				<b>Cp = 6.06</b>			
				Cp = Accuracy Spec/Expanded Unc			
Manufacturer's Precision Specification for this volume:							0.06 $\mu$
Manufacturer's Accuracy Specification for this volume:							0.2 $\mu$

Table 1. Uncertainty estimate of a pipette volume measurement.

(expanded) uncertainty. Guide 25 recommends reporting uncertainties at the 95% CI, which is k=2.

The gravimetric pipette calibration error budget, shown in Table 1, has major sections corresponding to the model describing the measurement system variables/errors that make knowing the true value uncertain. These include:

1. The balance which is used to measure  $m_1$  and  $m_2$ , the tare and gross, plus an estimate of the evaporation loss  $m_E$ . The balance sources of error include its reproducibility, linearity and resolution (rounding up or down). The manufacturer's specifications for the microbalance used for the gravimetric calibration can be used. If studies have been done to determine to estimate the uncertainty of balance measurements, they should be used. Table 1 uncertainty estimates are based on manufacturer's specifications and the evaluation of balance QC Data. These estimates are not absolute and have uncertainty. For example, the evaluation of the QC data from two different weight sets collected on 3 balances gave estimates that varied by up to 20%. Therefore, the average was used in the budgets. This information is shown in Figure 3 and discussed later.
2. Water temperature and evaporation rates contribute to the total uncertainty. Engineering estimates were made to define the interval of the uncertainty around the values. Various techniques can be used to minimize the

evaporation rates. Humidity traps are available from balance manufacturers as part of their pipette calibration kits. Other techniques involve keeping the room humidity near ~ 60% or pipetting into capable vials. Studies can be done to determine the evaporation rate variations.

3. Air density parameters of the Z factor are usually determined at time of calibration. However, some organizations have determined the range of variation of their humidity and temperature over a year from QC data and have used the range of barometric pressures over the year for their facility. In cases where tight controls are maintained on humidity and temperature, a constant Z factor can be used.
4. The coefficient of expansion for the pipetting device was borrowed from the Biohit PLC. error budgets found on their website at [www.biohit.com/pdf/app13.pdf](http://www.biohit.com/pdf/app13.pdf). [6] This value is much larger than the estimate given in the example of ISO/TC 20461. In correspondence with the Biohit Plc. authors, it was learned they chose a large value to be conservative. Their published budgets were the first available on the internet.
  - A correlation between the volume delivered and the coefficient was determined for 1 $\mu$ l and was multiplied by the size of the volume being calibrated. This was done for the Z factor components also. An

- engineering estimate of  $\pm 2^\circ$  K was picked for the heating of the pipette during calibration. This is an estimate as no studies were done on this variable.
- Dividing these component intervals by the appropriate number of standard deviations standardizes them. If the estimate has been derived from experimental data, a normal distribution is assumed and should be divided by the appropriate whole integer, if it has been expanded. If not, the standard deviation is used. Engineering estimates or data from calibration reports, manufacturer's specifications and other sources are assumed to come from rectangular distributions that have a probability of 1 and contains the true value. Therefore, the range should be divided by two and then be divided by the square root of 3 to obtain an estimate of the standard uncertainty.
  - These standard uncertainties have different units that must be normalized by converting them to the same unit. Multiplying them by the appropriate sensitivity coefficient does this. ISO/TR 20461 reminds the reader that uncertainty estimates do not require exact values. Therefore, using the approximation of 1 microgram ( $\mu\text{g}$ ) = 1 nanoliter (nl) to convert  $\mu\text{g}$  to nl is acceptable because the difference between them is small. After the standard uncertainties are multiplied by the sensitivity coefficients the standard uncertainties are all in the same unit.
  - These standard uncertainties are converted to variances by squaring them. The variances are summed and the square root taken to give an estimate of variation for the measurement system. An examination of them indicates the balance is the major source of uncertainty. Therefore the choice of balance and the choice of the number of places is of utmost importance.
5. The pipette's repeatability is usually the major source of uncertainty when considering all the variables in the calibration or verification. In addition to the mechanical operation and tip variation, the operator technique varies the volumes dispensed. The ISO/TR 20461 suggests the uncertainty of a volume measured by a pipette can be estimated by assuming the manufacturer's specification has a rectangular distribution with a probability of 1 in finding the volume dispensed within this interval. The pipette precision value used in Table 1 was based on the *average* standard deviation of 30 calibrations based on 10 measurements by different operators over several weeks. The precision estimate of 15.7 nl is 4 times larger than the measurement system standard deviation of 4.18 nl. Both the ISO Technical report and the draft ISO gravimetric calibration standard report the combined uncertainty of a gravimetric calibration system should be small compared to the precision of the pipetting. This has been confirmed by the author's experience. Smaller ratios of the calibration system uncertainty over the pipetting precision are seen for volumes  $< 50 \mu\text{l}$ .
6. Squaring, summing and taking the square root of these two components gives a standard uncertainty. In the example above the measurement system uncertainty only increases the pipetting precision from 15.97 nl to 16.51 nl or by  $\sim 3.4\%$ . This value is converted to  $\mu\text{l}$  by divided by 1000 nl/ $\mu\text{l}$ . The standard uncertainty of a volume measured by a pipette is usually expressed in  $\mu\text{l}$  or ml, not nl.
7. The GUM recommends all uncertainties be expressed at the 95% confidence level. Therefore, the standard uncertainty is expanded by a k factor of 2. Hence it is called the expanded uncertainty at  $k=2$ . The expanded interval of  $\pm 0.033 \mu\text{l}$  is the best estimate of the uncertainty of a single volume measured by the operator and should contain the true volume 95% of the time. The error budget gives documented evidence of the quality of measurements made to calibrate/verify a pipette's accuracy.
- The estimate of uncertainty on the final calibration report should be the uncertainty of the average volume delivered, based on the square root of the number of measurements made. Table 2 shows the uncertainty estimates based on average of 10 measurements as prescribed in the ISO draft standard. The uncertainty estimate provided by the calibration organization is based on their personnel and environmental conditions at time of calibration. The user needs to develop estimates of volume measurement uncertainty using their operators, in their environment, and on the material being pipetted, because they will be different than the calibration organization's.
  - The precision estimate from the pipetting is reduced by the square root of the 10 measurements. The standard deviation of the mean is 5.05 nl. It is now only 20% larger than the calibration system's combined uncertainty. Note the expanded uncertainty of  $0.013 \mu\text{l}$  is now less than half the  $0.033 \mu\text{l}$  uncertainty of a single measurement with the pipette.
8. The author's error budgets in Tables 1 and 2 contain one more piece of information. It calculates the ratio of the manufacturer's accuracy tolerance and the expanded uncertainty. This is a type of measurement system capability indices. It is desirable to have the measurement system uncertainty less than 1/3 of the tolerance. Another way of looking at this is to divide one side of the tolerance by the uncertainty to give the number of uncertainty intervals inside the tolerance. Ratios greater than 3 are very good. The ratio of the manufacturer's specification over the expanded uncertainty of the average calibration accuracy is 15.3 : 1. This calibration system uncertainty is more than adequate for calibrating pipettes.

Pipet Calibration Uncertainty Budgets							
Pipette: L20, 20 ul						20	20 ul
Balance UMT (7 places)							
Parameter	Interval	Distribution	(u) Standard	(c) Sensitivity	units	units	units
	+/-	units	1.73205081	Uncertainty	units	Coefficient	c*u (x)
<b>Balance</b>	Uncertainty	5.68 ug	Normal	2.84 ug	1 nl/ug		2.84 nl
	Linearity	1 ug	rectangular	0.58 ug	1 nl/ug		0.58 nl
1st Value	Reproducibility	0.25 ug	rectangular	0.14 ug	1 nl/ug		0.14 nl
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1st Value	Resolution	0.1 ug	rectangular	0.06 ug	1 nl/ug		0.06 nl
2nd Value	Resolution	0.1 ug	rectangular	0.06 ug	1 nl/ug		0.06 nl
	Temp. Drift	1.0 ug	rectangular	0.58 ug	1 nl/k		0.58 nl
Correction for Evaporation Loss		5 ug	rectangular	2.89 ug	1 nl/ug		2.89 nl
<b>Water</b>	Temperature	0.2	rectangular	0.12 K	4 nl		0.46 nl
<b>Air</b>	Temperature	1 K	rectangular	0.58 K	0.09 nl/K		0.05 nl
	Pressure	27 hPa	rectangular	15.59 hPa	0.024 nl/hPa		0.37 nl
	Relative Humidity	10 %	rectangular	5.77 %	0.002 nl/%		0.01 nl
<b>Pipette</b>							
Cubic Expansion Coefficient	9.00E-05 K-1	rectangular	0.0001		-40000 nl*K		-2.08 nl
Temperature	2 K-1	rectangular	1.15		0.02 nl/K		0.02 nl
<b>Standard Uncertainty Associated with the Volume V<sub>20</sub> Measured gravimetrically</b>							<b>4.18 nl</b>
<b>Average Standard Deviation of the Mean of 10 measurements divided by sq. rt. Of 10</b>							<b>5.05 nl</b>
<b>Combined Standard Uncertainty of the Calibration</b>							<b>6.56 nl</b>
<b>Converted to microliters =</b>							<b>0.007 ul</b>
<b>Expanded Uncertainty @ k = 2</b>							<b>0.013 ul</b>
				Cp =	15.3		
				Cp =	Accuracy Spec/Expanded Unc		
Manufacturer's Precision Specification for this volume:							0.06 ul
Manufacturer's Accuracy Specification for this volume:							0.2 ul

Table 2. Uncertainty of a gravimetric calibration's average volume.

## Sources of Uncertainty and Their Magnitude

ISO/DIS 8665-6 specifies the use of analytical balances that conform to the International Organization of Legal metrology recommendation, OIML R 76-1. The scale graduation value of the balance used for testing should be chosen according to the selected volume of the apparatus under test. A table in the draft gravimetric standard lists the minimum requirements for balances to be used for various volume calibrations. Balances have random and systematic errors.

In developing error budgets, manufacturer's specifications are often used in estimating a balance's uncertainty. However, it states "If the standard uncertainty of measurement of the balance is known (e.g. from the balance calibration certificate), this may be used instead of the repeatability and linearity. The standard uncertainty of measurement should not be more than 2 to 3 times the readable graduation."

The authors have studied the precision of several balances and evaluated the data from various balance quality assurance programs and found there is a large day-

to-day source of variation in balance measurements.[7] The balance standard deviation for weighing the same weight is often two or three times the manufacturer's specification for repeatability. Better than an uncertainty estimate based on a calibration certificate or manufacturer's specification, is one that is based on the evaluation of a balance measurement control program.[8]

A study was done to determine the uncertainty of the balances based on weighing masses comparable to the volumes of water that would be weighed during a calibration. Three microbalances were evaluated daily with two weights for both of the 7 and 6 place ranges. Two different sets of weights were used during the year. One was the primary set, which was only used while the daily set of weights was sent out for calibration. Most of the data came from measurements of the daily set. Uncertainties were determined for the balances, based on the weighing process variation, the uncertainty of the standards and half of the observed bias, based on the apparent mass value on the calibration certificates. In all cases, the process variation was the major source of uncertainty. The estimates of uncertainty varied by 10 to 20%. The data are summarized in Figure 3 which gives

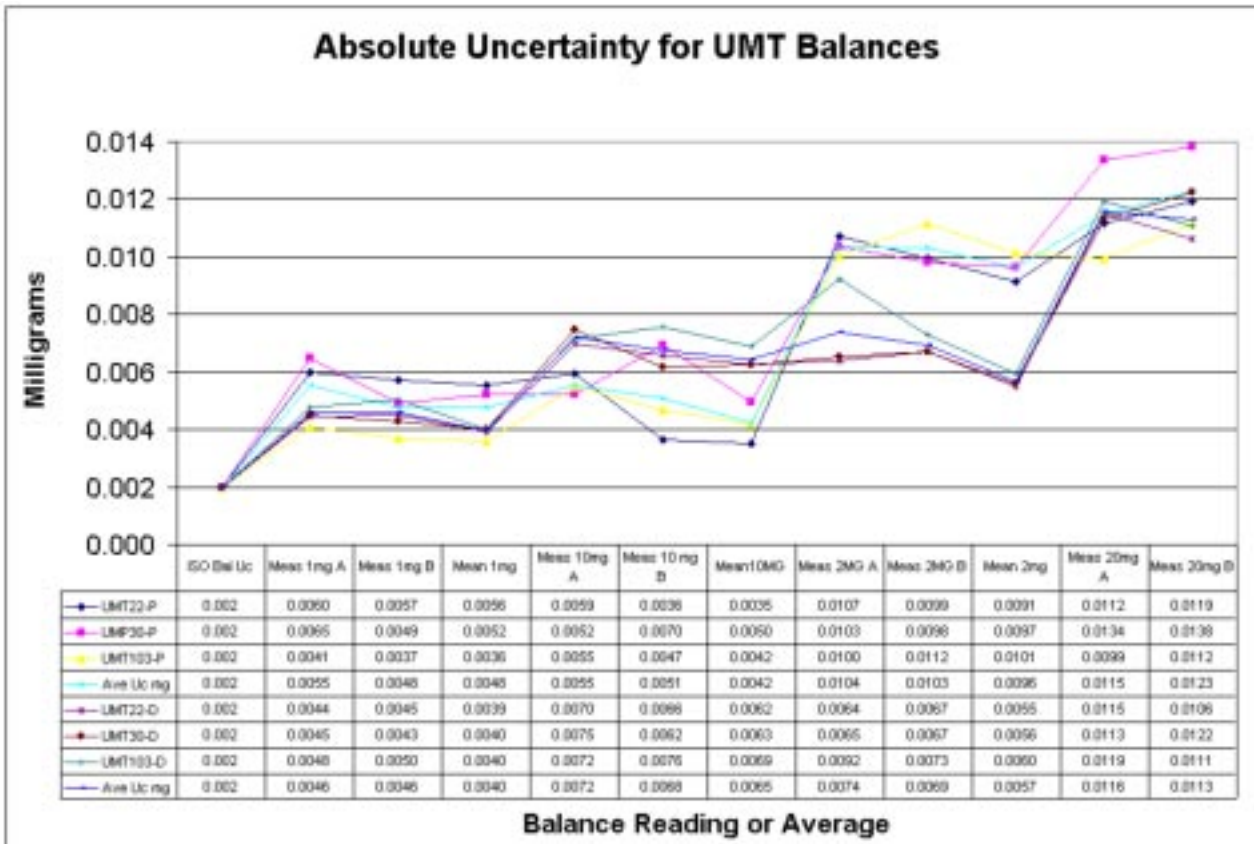


Figure 3. Uncertainty estimates of 3 UMT balances using 2 sets of weights.

the ISO/DIS recommended uncertainty of 0.002 mg for volumes of 10 µl and below. The absolute uncertainty increases with the size of the standards from 1 to 20 mg. The 1 and 10 mg weights were weighed on the 7-place scale and the 2 and 20 mg weights were weighed on the 6-place scale. The calculated uncertainties are all larger than the manufacturer’s specifications.

Evaluation of the errors associated with the balances indicate the requirements recommended by the committee members that drafted ISO/DIS 8655-6 underestimate the uncertainty of electronic balance measurements. This is especially true for small volume calibrations.

The primary value of generating error budgets is their use as tools in determining where to make the most cost-effective process improvements.

### Photometric Pipette Calibrations

The photometric method of calibrating pipettes has shown significant growth and improvement in recent years. The authors evaluated this method of calibrating pipettes to determine its capability to meet in-house accuracy and precision requirements. The system is

shown in Figure 4 and is composed of a spectrophotometer, printer, reagent kits, calibration standards and blanks, etc. The method offers several advantages over the conventional gravimetric method. The major one being the time required to calibrating a pipette. Photometric calibrations routinely took less than 20% of the time required using the author’s gravimetric system. Calibrating a 1µl syringe from 0.1 to 1 µl took less than 10% of the time required doing it gravimetrically. The printouts from the actual calibration provide all of the basic data required for most QA systems.

The identification of the pipette, operator and instrument serial number, the date, time, temperature, measurements, statistics, etc. are recorded plus the date of last calibration, software version, and reagent lot number. The documentation is satisfactory for paper recording keeping systems. The system can be interfaced to a computer system to provide electronic records.

The QA is comprehensive. The reagents have stated shelf lives. Each reagent blank will accommodate from 1 to 22 volumes of dye that are measured into it from the pipette during calibration. The reagent cost per measurement is from \$0.50 and higher, depending on



Figure 4. Artel PCS®3 Pipette Calibration System.

which of the 6 ranges of dye are being used and the volume being measured. The reagent costs are progressively higher for the larger volumes. The volumes that can be calibrated range from 0.1 to 5,000  $\mu\text{l}$ .

### Comparison of Calibration Methods

Many pipettes were calibrated using both the spectrophotometric and gravimetric methods. The precisions reflect the operator's ability to pipette and the variation of the calibration system. They were usually comparable. About 5% of the time, the average calibrated volume of a vial would be 0.5 to 0.8 % higher than the other averages on different vials. This could be due to evaporation. However, all of the observed values on the vial to vial comparisons fell within manufacturer's specifications.

Comparisons of measurements made at different times were affected by many variables. A test was designed that compared the same volume pipetted with the same pipette under the same environmental conditions. A vial of dye was placed on the pan of a 5 place Mettler AX205 analytical balance interfaced to a computer using Mettler Balance Link version 2.2 to record the net weight of dye removed by a Rainin EDP2 100  $\mu\text{l}$  digital pipette. The first stable reading was recorded in the spreadsheet and the reading from the PCS®3 printer was recorded. The vial was zeroed and another volume of dye was removed, the first stable net weight was sent to the next cell in the spread sheet and the dye was dispensed into the reagent vial and read. This process was repeated 11 times.

The summary statistics of 10 calibrations are shown in Figure 5. There was no statistically significant difference in the average biases or standard deviations for the 10 sets of calibrations, even though there were significant differences between individual samples in both methods. Both methods are capable of providing calibrations with

uncertainties that are well within customer tolerances.

### Spectrophotometric Calibrations

The vendor provided information showing traceability to the national measurement system for all of the instruments used in calibrating the spectrophotometers, preparing the reagents, monitoring the environmental conditions, and validating the spectrophotometric system against the gravimetric method. An example of the uncertainty budget for the Artel PCS®3 Pipette Calibration System is shown in Table 3. The main components of the system are the photometric, temperature, mixing, reagents, stability, packaging and physics.

The instrument uncertainty addresses the errors for the two wavelengths the vial are read and zeroed. It addresses imprecision and non-linearity, vial imperfections, The root sum square of these error sources has a relative uncertainty of 0.24%. The reagent uncertainty components contribute another 0.25% and are detailed in the table.

The manufacturer has identified the basic measurement units of the system variables, defined the probability distribution they came from and the range of the variables. Next, they are converted into standard uncertainties using the appropriate sensitivity coefficient to convert them to percents, squared them, combined the variances and took the square root to estimate the standard uncertainty for the parameters combined. The table also shows the total combined and expanded uncertainties. The manufacturer has claimed a much larger uncertainty than calculated. This allows for customer pipetting variation.

The authors did not find any pipettes that exceeded the claimed accuracy of the method that were not confirmed by the gravimetric method.

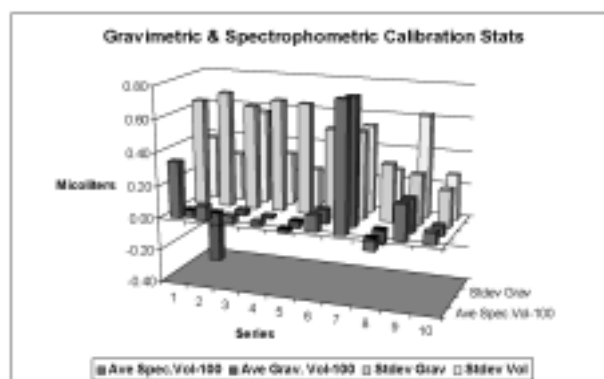


Figure 5. Comparison of gravimetric and spectrophotometric average biases.

ERROR BUDGET FOR PCS RESULTS								
(example shown: 10 th delivery at 50 uL, range 2 solution)								
	Probability Distribution	Range of Values +-	Standard Uncertainty	Unit	Sensitivity Coefficient	Unit	Weighted Variance	Relative Uncertainty
<b>Instrument Uncertainty</b>								
photometric uncertainty								
due to wavelength error at 520 nm	rectangular	0.40	0.25	nm	0.90	%/nm	1.9291E-06	0.14%
due to wavelength error at 730 nm	rectangular	1.00	0.50	nm	0.10	%/nm	3.3333E-07	0.06%
due to zero error at 520 nm	rectangular	0.00005	0.00003	Abs	1	%Abs	9.3333E-10	0.00%
due to zero error at 730 nm	rectangular	0.00050	0.00020	Abs	0.989	%Abs	6.8881E-08	0.03%
due to imprecision of measurement at 520 nm (worst case)	normal	0.00003	0.00003	Abs	33.3	%Abs	9.9999E-07	0.19%
due to imprecision of measurement at 730 nm	normal	0.00003	0.00003	Abs	0.989	%Abs	7.4385E-10	0.00%
due to nonlinearity of photometric response at 520 nm	rectangular	0.0010	0.00050	Abs/Abs	1.00	%Abs/Abs	3.8882E-07	0.06%
due to nonlinearity of photometric response at 730 nm	rectangular	0.0100	0.00500	Abs/Abs	1.00	%Abs/Abs	3.8882E-07	0.06%
due to vital imperfections (causes error in T30 zero)	rectangular	0.00200	0.00115	Abs	0.989	%Abs	1.1019E-06	0.13%
temperature uncertainty	rectangular	0.50	0.29	deg C	0.185%	/deg C	2.2886E-07	0.05%
incomplete mix of sample and blank	normal	0.10	0.06	%	1	%/%	3.3333E-07	0.06%
<b>Total Relative Standard Uncertainty of the Instrument</b>			<b>0.24%</b>				5.7610E-06	
<b>Reagent Uncertainty</b>								
Lot to Lot inaccuracy (error in cal codes)								
due to imperfect tracing of balance to national standards	rectangle	5.48E-06	3.12E-06	gram	1	%g	9.7290E-10	0.00%
due to balance drift, inaccuracy, or imprecision	rectangle	5.00E-06	2.89E-06	gram	20	%g	3.3333E-07	0.06%
due to uncertainty in comparison method, PCS to Grav (eg due to imperfectly compensated evaporation)	rectangle	1E-04	5.77E-05	gram	20	%g	1.3334E-06	0.11%
due to error in dilutions	rectangle	5E-03	2.89E-03	gram	0.2	%g	3.3333E-07	0.06%
Degradation of reagents in storage	rectangle	0.20	1.19E-01	%	1.00	%/%	1.4662E-06	0.12%
Uncertainty in blank volume								
fill error	rectangle	7.18E-03	4.13E-03	gram	0.2105	%g	7.5691E-07	0.06%
subsequent loss of liquid in storage	rt triangle	4.19E-03	2.74E-03	gram	0.2105	%g	3.3321E-07	0.06%
Color variations of glass vials (causes error in T30 zero)	rectangle	0.301	5.77E-04	Abs	0.989	%Abs	2.7544E-07	0.05%
Non adherence to Beer-Lambert Law (deviation of slope from ideal)	rectangle	0.2	1.19E-01	%	1.00	%/%	1.5553E-06	0.12%
<b>Total Relative Standard Uncertainty of the reagents</b>			<b>0.25%</b>				6.4143E-06	
<b>Combined Relative Standard Uncertainty</b>			<b>0.35%</b>				1.2176E-05	
<b>Overall Uncertainty with coverage factor of 2</b>			<b>0.70%</b>					
<b>Claimed Accuracy of Method</b>			<b>1.00%</b>					

Table 3. Uncertainty budget for the Artel Pipette Calibration System.

## Conclusions

The calibration of pipettes requires a reliable method and knowledge of the sources of error that can affect the quality of the measurements made during the calibration. Knowledge of the measurement system uncertainty will allow the calibrating organization to select the method that will provide cost-effective measurements that meet their required accuracy or error tolerances. Error budgets for a measurement system provide useful information in evaluating the adequacy of the measurements and to identify the most significant areas for improvement.

## References

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John P. Clark, A. Harper Shull, Westinghouse Savannah River Company. Tel 803-725-3654 & 803-952-4687, johnp-clark@srs.gov, harper-shull@srs.gov.

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