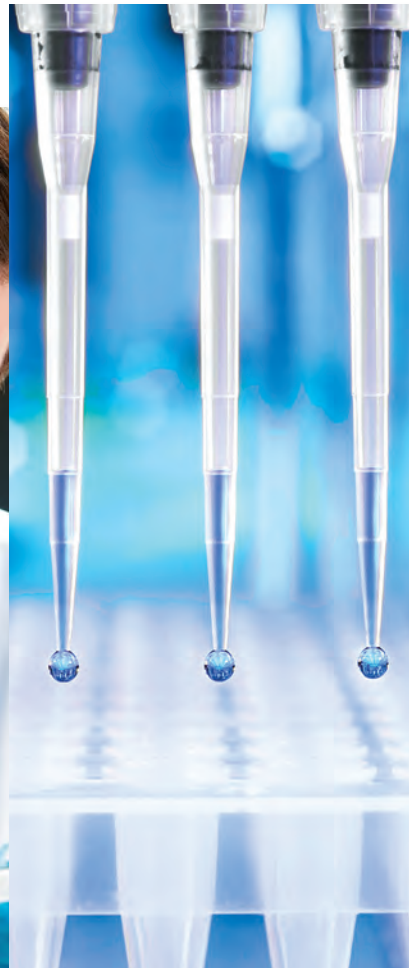


# Artel Lab Reports

*A series of publications that provide technical information regarding the use, application, and metrology related to liquid handling instrumentation.*



# Artel Lab Reports

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# Artel Lab Report

This publication provides technical information regarding the use, application, and metrology related to liquid handling instrumentation.

## How many Data Points

“I’m a bit of a freak for evidence-based analysis. I strongly believe in data.”

Sir Gus O'Donnell

### Human beings are creatures of habit

We often seek stability and continuity and are wary of change. However, inspection, evaluation, and subsequent change are often necessary for growth and improvement, both inside and outside the laboratory. A worthy project for your laboratory to undertake this year may be to evaluate your pipette checking routine. One important element of the procedure is the number of data points that are taken for each pipette at each volume setting. Since the needs of each laboratory vary widely, it is difficult to give a universal answer to the question. If your laboratory can tolerate a high margin of error, then you can afford to take fewer data points. As your demand for excellence increases, so does your number of data points.

### The issue of confidence

A quantitative answer to the “how many data points” question boils down to the issue of confidence. How certain do you want to be that your pipettes are functioning within established limits? Let’s step through a process which can help you evaluate your laboratory’s needs.

### What do the standards say?

Ten replicates at each volume are specified by ASTM<sup>1</sup> ISO<sup>2</sup>, and CLSI<sup>3</sup> standards, and are suitable for testing trueness and precision. According to ASTM four replicates may be used as a quick check for trueness alone.

### How do you use your pipettes in actual service?

Most laboratories have a number of different applications for pipettes, each with its own requirements. For simplicity’s sake, however, a single pipette testing procedure is often used for all pipettes. If this is the case, then you need a procedure that is adequate for all applications. Choose your most demanding application and design your testing procedure around it. For this application, determine upper limits for percent systematic error ( $SE_U$ ) and percent precision ( $CV_U$ ). These are limits that you would expect the pipette to exceed only rarely.

### Know your limits.

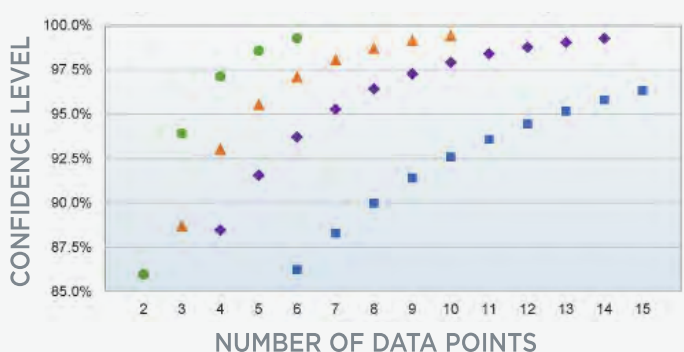
It is very important that your test procedure for trueness and precision delivers results well within your upper limits. Choose test limits ( $SE_T$  and  $CV_T$ ) which you can meet consistently, given a reliable pipette.

### Establish critical values using the following formulas:

Critical value for Trueness  
 $CV_{SE} = (SE_U - SE_T) / RE_T$

Critical value for Precision  
 $CV_{RE} = RE_T / RE_U$

Figure 1. Number of data points required for trueness testing



■ CRITICAL VALUE = 0.5    ◆ CRITICAL VALUE = 0.75    ▲ CRITICAL VALUE = 1.0    ● CRITICAL VALUE = 1.5    USE CRITICAL VALUES FOR TRUENESS (CVSE)

## Establish a confidence level for your testing.

This is the probability that a pipette which performs at the testing limit is better than the upper limits for trueness and precision. Many laboratories require a confidence level of 95%.

## What pipette qualities are you testing... trueness and precision, or precision alone?

The number of data points for trueness testing may be different from that required for precision. If testing for both trueness and precision, use the greater of the two results.

## Finally, use the charts...

to find the number of data points required to assure the confidence level you require.

## Example

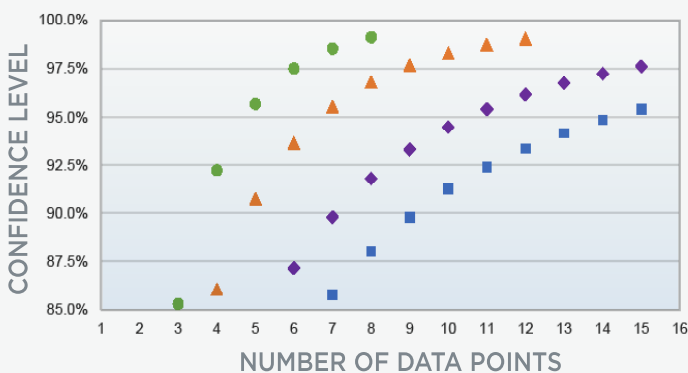
Suppose that your most demanding applications cannot tolerate systematic error greater than 3% ( $SE_U$ ) or random error greater than 2% ( $RE_U$ ). Based on your history of pipette testing data you are reliably able to achieve systematic error below 2% ( $SE_T$ ) and random error below 1% ( $RE_T$ ) when testing good pipettes, so these are the limits you impose on all pipettes tested. You require a confidence level of 95%.

Calculate the critical value for trueness =  $(3\% - 2\%) / 1\% = 1.0$ . Determine the number of data points required from Figure 1, using the orange triangles (critical value = 1.0) at the 95% confidence level. You find that 5 data points are needed to satisfy the trueness testing requirement. By taking 5 data points and maintaining a systematic error of less than 2%, you establish with a 95% confidence level that the pipette is within the 3% upper limit for systematic error.

In a similar way, the critical value for precision  $CV_{RE} = 1\% / 2\% = 0.5$  and the number of data points required is read from Figure 2, using the orange triangles (7 data points to surpass the 95% confidence level).

Overall, you need to take at least 5 data points to satisfy both the trueness and precision requirements.

Figure 2. Number of data points required for precision testing



■ CRITICAL VALUE = 0.65    ◆ CRITICAL VALUE = 0.6    USE CRITICAL VALUES FOR PRECISION ( $CV_{RE}$ )  
 ▲ CRITICAL VALUE = 0.5    ● CRITICAL VALUE = 0.4



### Q: Who should check our pipettes?

**A:** Often, the operator has as great an influence on results as does the pipette itself; different pipetting techniques or conditions can lead to errors of more than 5%<sup>6</sup>, even when the pipette is functioning correctly. For many laboratories, this factor alone will put results outside of the desired tolerance. The most reliable way to assure that your laboratory's results are correct is to have each operator check his or her own pipettes on a regular basis.

### Q: If a pipette fails its performance check, but is then retested and passes, can it be put back in service without further concern?

**A:** No, not necessarily. While it is possible for a properly working pipette to infrequently fail a performance check, it is likely that this failure points to a faulty pipette, a poor procedure, or operator error. At the very least, the pipette should be retested more thoroughly, for example with more data points.

### Q: If a pipette's delivery is generally good, but some data points seem out of line, is it OK to discard them in order to be able to pass the pipette?

**A:** The fact that some data points are not within an expected tolerance range points to a problem which should not be ignored. The only exceptions should be data points for which a defensible reason exists why the data point can be discarded. At the very least, the pipette should be retested using more stringent criteria. If it continues to display this behavior, then it needs to be repaired or replaced.

If you wish to calculate Critical Values other than those shown on the charts, use  $t/\sqrt{n}$  for trueness critical values, and  $1/\sqrt{F}$  for precision critical values.  $t$  is obtained from Student's one tailed distribution, and  $F$  from the one tailed  $F$  distribution, tables of which are available in statistics reference<sup>4,5</sup>. For the precision calculation, it is assumed that the Upper Limit is based on a test using 30 data points.

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# Artel Lab Report

This publication provides technical information regarding the use, application, and metrology related to liquid handling instrumentation.

“...a small mistake in pipetting can cause a large error in the final result. It is, therefore, of great importance to evaluate and to reduce, wherever possible, both random and systematic errors in liquid sample handling...”<sup>7</sup>

Sari Ylätupa, PhD,  
“Choosing a Pipetting  
Technique Affects the  
Results of Your Analysis,”  
European Clinical  
Laboratory

## Impact of Pipetting Technique

### The pipette is a reliable precision instrument

that has been used and trusted for many years. However, as with many forms of instrumentation, a pipette will perform only as well as the operator's technique allows.

Differences in technique – some more than others – can alter delivery volumes. With increasing demand for accuracy, quality and productivity the importance of understanding and developing optimal pipetting technique becomes imperative. Let's review the results of a study conducted at Artel to determine these differences and assess their impact upon pipetting accuracy.

### Method & Procedure

The reference pipetting method used in each of these experiments was as follows: The pipette mechanism was “warmed up” by gently depressing and releasing the plunger 15 to 20 times. The pipette tip was prewet by aspirating and dispensing an aliquot of the sample solution three times<sup>1</sup> With the plunger depressed to the first stop, the

tip was immersed approximately one millimeter into the sample solution and held there for a half second. The aliquot was aspirated from the sample solution by gently releasing the plunger, keeping the tip in the sample solution for two seconds before removal.

The aliquot was delivered by placing the pipette tip on the side of a glass vial at a 45° angle just above the meniscus and slowly depressing the plunger past the first stop to deliver the entire aliquot.

Each experiment consisted of two runs of ten data points each, using an adjustable 20 µL Eppendorf manual action air displacement pipette<sup>†</sup> set at 5 µL and Eppendorf disposable pipette tips, on the Artel PCS<sup>®</sup> Pipette Calibration System. Each experiment was performed as a comparison of one pipetting technique versus another.

The results for each pair of techniques were compared concerning trueness and precision. The measurement used for rating the precision of each technique was the coefficient of variation (%CV). Trueness was defined as the percent difference in mean delivery volumes between the two pipetting techniques.

*<sup>†</sup>In an air displacement pipette, many sources of pipetting error are magnified by the ratio of “dead air” above the liquid level to the liquid volume in the tip.<sup>2-6</sup> In conducting these experiments, a 20 µL pipette was used to deliver 5 µL, so the amount of dead air was increased, compared to pipetting 20 µL with the same pipette.*

### Eliminating sources of error

#### Don't leave your tips high and dry

The greatest discrepancies observed during this study were the differences between dry and prewetted pipette tips. Dry pipette tips consistently delivered significantly lower volumes than did the prewetted tips, a fact which other researchers have noted.<sup>2-6</sup> No difficulty with precision was observed using either prewetting or no prewetting. However, differences in trueness of up to 7% between runs using dry and prewetted tips were noted while using the 20 µL pipette set at 5 µL. Additional experiments using a 250 µL pipette set at 25, 50, 100 and 250 µL consistently showed differences in the trueness of the volume pipetted of up to 2%.

Prewetting the pipette tip influenced trueness by increasing the humidity within the tip, thus minimizing evaporation of the solution. Similarly, increased ambient humidity minimized evaporation. The beneficial effect of prewetting was less significant with high ambient humidity. Ambient humidity for these runs was 50%.

## Not too hot... not too cold...

Variation in the temperature of the solution being pipetted was observed to be the second largest cause of pipetting error in this study. Three sample solutions were brought to three different temperatures: 8.5, 25 and 30 °C. Solutions that were warmed to 30 °C consistently delivered lower volumes than room temperature samples. Similarly, solutions that were cooled to 8.5 °C delivered higher volumes than the ambient (25 °C) sample. The differences observed in this study were significant, ranging from 3 to 7%. Figure 1 below shows the effect of sample temperature on pipetting trueness.

In addition to trueness problems seen with samples that were not at room temperature, there was some difficulty in obtaining good precision. Runs typically produced between 0.5% and 1.0% CV, although the CV went as high as 3.6% in some cases. As the solution was allowed to approach room temperature, the precision of the results improved. Similarly, if the pipette end or tip was warmed, even just by casual handling, differences in delivery volumes were observed. These differences and inconsistencies were smaller and less significant than those resulting from variations in solution temperature.

## Shift out of Reverse

Reverse mode pipetting is a method of pipetting commonly used with viscous liquids. This is a technique in which the plunger is depressed past the first stop to aspirate the aliquot from the sample, and depressed only to the first stop to deliver the aliquot. Reverse mode pipetting can make obtaining accurate results (for the pipetting of solutions similar to water in terms of density and surface tension) more difficult. The study showed that a typical precision for reverse mode was 1.4% CV. Differences in volumes delivered by standard and reverse mode techniques ranged up to 5%. The reverse mode consistently delivered a higher volume than the standard method of pipetting.

## Conclusions

Techniques among pipette users vary with background, personal preferences, and training. These differences in execution can affect the accuracy, precision and trueness of results being released from the clinical or research lab. To ensure pipetting accuracy, facilities should adopt standard operating procedures for pipetting techniques and ensure that all operators are trained to an adequate level of proficiency. By increasing the level of consistency in results obtained, the level of quality and credibility of the facility will be enhanced.



### Q: What are other techniques which can affect accuracy, precision and trueness?

A: Other error causing techniques<sup>8</sup> include:

- prolonged delay between aspiration and removal of the tip from the sample
- dragging the tip along the side of the container when the tip is exiting the sample
- variations in the size and shape of the sample container, and
- rate of plunger depression and release.

### Q: What impact will these techniques have upon my results?

A: Individually none of these factors resulted in an error greater than 2%. Cumulatively, however, two or more of these sources of error (e.g., prolonged delay and rate of plunger depression) could affect delivered volume significantly.

### Q: What factors other than technique differences can affect my results?

A: Component failure (e.g., a plunger seal or corroded piston), incorrect pipette tip, or incorrect installation of the tip can also affect your results.

**Figure 1. Effects of sample temperature on pipetting trueness**

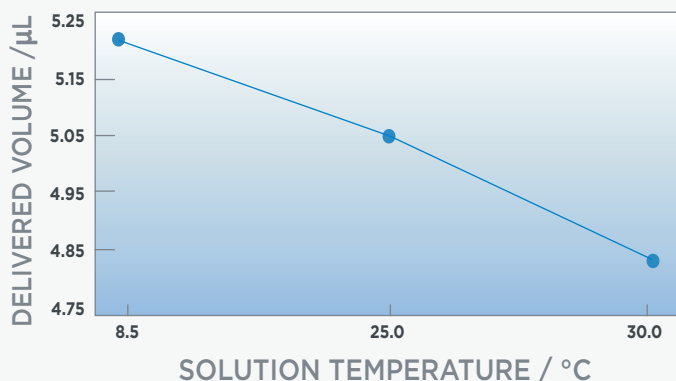


Figure 1 Experiment showing the effect of sample temperature on the trueness of pipetting results, using a 20 µL adjustable air displacement pipette set at 5 µL. Ambient temperature 25.0 °C.

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This publication provides technical information regarding the use, application, and metrology related to liquid handling instrumentation.

“Laboratory technicians who work in biosafety cabinets and use pipettes are at risk for developing musculoskeletal injuries and illnesses.”<sup>1</sup>

Ergonomic and epidemiologic evaluation of a biological laboratory.  
McGlothlin-J; Hales-T

## Pipette use and Ergonomics

### Any work environment including the laboratory,

can be a source of ergonomic stress. Laboratorians that use pipettes frequently may find not only their health, but their proficiency at risk. Two factors in pipetting which can cause the user physical stress are the design of the pipette and the manner in which it is used. An ergonomically designed pipette should pose few if any difficulties or risks to the user who, trained in body mechanics, knows which positions and postures to avoid. Awkward postures and repetitions are not bad in themselves, but can pose a problem if stresses are cumulative and tasks are not balanced.

Selecting your pipettes and the manner in which they are used is a relatively simple project which requires little more than applying some useful information. The investment of some research and training is far outweighed by the return: improved safety and health in the workplace, improved productivity, reduced absenteeism and turnover, and reduced probability of accidents and errors.<sup>3</sup>

### Potential problems

Manual action pipettes, one of the most commonly used laboratory instruments, can cause muscle

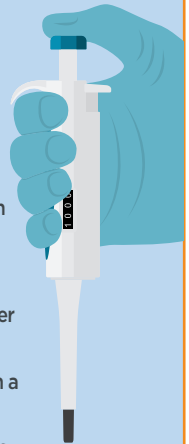
strain or tendon swelling, particularly if good body mechanics are not applied while pipetting. Manual pipetting involves several ergonomic stresses of the wrist, arm, and shoulders. The stresses are typically caused by repetition, awkward posture, and the excessive use of thumb force<sup>4</sup> when dispensing a sample. In addition, pipetting is done in a position where the thumb is not stable but nevertheless has to work to stabilize the grip around the pipette and to press down the plunger button of the pipette. Therefore the muscles have to work

as both mobilizing and stabilizing structures and are subjected to increased stress.<sup>5</sup>

These physical stresses are further aggravated by the mental pressure resulting from the accuracy and timing demanded in many pipetting procedures.<sup>2</sup> Taken together, these factors put laboratory technologists at a great risk of developing a **cumulative trauma disorder (CTD)**: one of a group of health disorders affecting the muscles, tendons, joints, and nerves, which can cause pain and swelling.<sup>3</sup>

### Steps that you can take to reduce the risk of developing a cumulative trauma disorder include:

- Rotate pipetting activities.
- Use only the force necessary to operate the pipette; do not use excessive force.
- Choose pipettes requiring less pressure.
- Use shorter pipettes, which allow for decreased arm elevation and thus eliminate the use of awkward postures.
- Consider using electronic pipettes, which are programmable and reduce the need for excessive thumb force and repetition.
- Use low profile waste receptacles for used tips. Receptacles should be no higher than the tops of the tubes being filled.
- Take short pauses of several seconds when you are unable to take a longer break.
- Use adjustable chairs or stools with built-in solid foot rests.
- Keep materials within reach. This allows your arms to remain close to your body which will reduce shoulder strain.
- Use multichannel pipettes for highly repetitive jobs.
- When pipetting in a standing position, anti-fatigue mats offer cushion between you and the floor.
- Align your ears, shoulders and hips to ensure your body is in a neutral position.
- Keep wrist in a neutral position, as if you were shaking hands.



# An informed technologist does not have to be a CTD statistic. With CTDs, protection and prevention are the best medicines.

## The solutions

The design of a pipette is as important as the manner in which it is used. Pipette manufacturers recognize the benefits of an ergonomically designed pipette, and this is revealed in the designs of pipettes on the market. For example, pipettes with a curved hilt allow a relaxed grip and reduce muscle strain.

Separate buttons for tip ejection, typically allow the operator to use a shorter, less forceful motion for tip ejection than do pipettes with a single “combination” button for both sample dispensing and tip ejection. This can reduce the stress on an operator’s thumb.

Manual action pipettes, however, require the user to relocate the thumb to another button, further stressing the muscles.

Several other features may make pipetting less stressful.

Non-slip, contoured surfaces reduce fatigue by increasing friction, allowing the use of a relaxed grip.

Some plunger buttons can be shaped with sloped or rounded surfaces to better fit the user’s hand. Other pipettes are lighter in weight and require less force to operate the plunger. Consider these features when purchasing and using your pipettes.

Avoiding CTDs is a simple and relatively inexpensive task, the importance of which cannot be overestimated. A healthy technologist will have better attendance, a better attitude, and better accuracy than one who is coping with the effects of CTDs. Pipetting results are only as reliable as the mechanism (operator + environment + pipette) with which they are obtained. A laboratory’s technologists, as well as its pipettes, should be in sound condition, and should be in a comfortable, controlled environment.

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### Q: What are some of the more common cumulative trauma disorders, and what are their symptoms?

**A:** Among the more common forms of CTD are DeQuervain’s disease and carpal tunnel syndrome, in which the median nerve running through the wrist becomes compressed. Symptoms of carpal tunnel syndrome include pain, numbness, or tingling in the first three fingers and the base of the thumb.<sup>3</sup> DeQuervain’s disease affects the tendons on the side of the wrist and at the base of the thumb. Symptoms include pain and difficulty in movement.<sup>3</sup>

Other CTDs include epicondylitis, commonly referred to as “tennis elbow,” which is an inflammation of the tendons within the elbow. Symptoms include swelling, pain, and weakness. Tendinitis is an inflammation of the tendon in the wrist and hand which can cause swelling and pain. “Trigger finger” results when a tendon sheath in the finger swells and becomes locked. The condition is referred to as “trigger finger” because attempts to move the finger result in a snapping and jerking movement.<sup>3</sup>

### Q: How do I determine my risk of developing a CTD?

**A:** “Yes” answers to any of the following indicate that CTD risk factors are present at your job:<sup>6</sup>

- Are there frequent, repetitive motions?
- Does your working position require bending of the neck, shoulder, wrist, or finger joints?
- Are there forceful or quick, sudden motions?
- Do you work across the midline of your body or out to the side?

### Q: What should I do if I think I have a CTD?

**A:** Talk to your supervisor, request referral to health and safety professional at work, or see your doctor. Remedies, such as acetaminophen or ibuprofen, are often helpful.<sup>7</sup> However, as with any health concern, consider seeing your physician, who can accurately assess your condition and recommend an appropriate course of action, which might include physical therapy to help heal strained muscles.

### Q: Are there any additional safety considerations, not necessarily mechanical or ergonomic in nature, that I should keep in mind when working with pipettes?

**A:** Pipetting should never be done by mouth, even if extension tubes are used, in the event that liquids or vapors are drawn into the body through the mouth or nose.<sup>8</sup>

Broken glass pipettes can also pose a danger to users. Unusable broken glassware or pipettes should be collected in a suitable sharps container which can be sealed for disposal when full.<sup>9</sup>



# Artel Lab Report

This publication provides technical information regarding the use, application, and metrology related to liquid handling instrumentation.

“ I welcome new words, or old words used in new ways, provided the result is more precision, added color or greater expressiveness.”

William Safire

## Defining Accuracy, Precision & Trueness

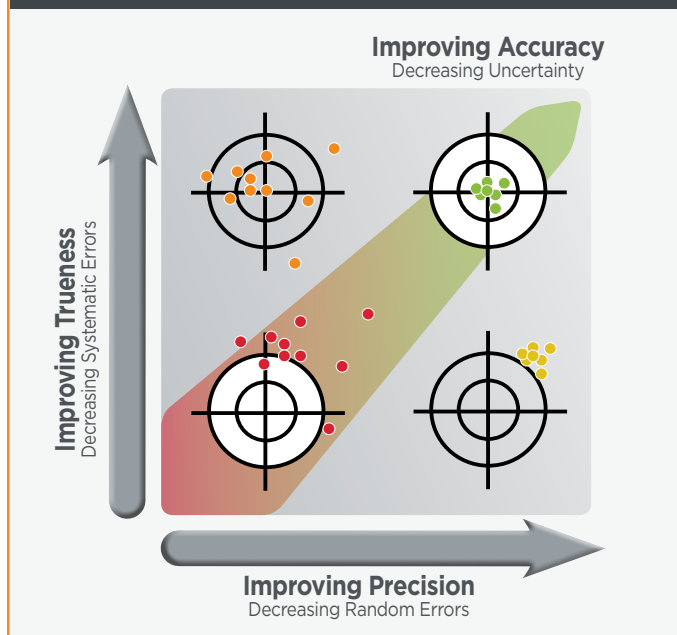
### Language influences thinking

and careful use of words when evaluating data allows us to understand how best to improve laboratory accuracy. Accuracy is a particularly important concept because it is foundational to the quality of laboratory measurements. Accuracy invokes an image of something that is correct, reliable and trustworthy. As a conceptual goal, accuracy is universally sought after.

However, what exactly do we mean when we speak of accuracy? Try this experiment: The next time someone uses the word “accuracy”, engage in a conversation and try to understand as clearly as possible what they mean by the term. These are some possibilities:

- Highly consistent results (e.g., a low standard deviation, or small CV).
- An average which is very close to the true value.
- Knowing that each measurement correctly represents what is present in the sample.

Figure 1. Accurate results are achieved by improving both precision and trueness.



### Removing ambiguity

Historically, there have been two different ways that the word “accuracy” is used in the context of liquid handling. We can use this word to describe a single liquid delivery, and also how the average value of a group of dispenses can be evaluated for accuracy. This “double usage” of the term is still common today, but recent work in developing definitions for ISO IWA 15<sup>1</sup> (based on the vocabulary of metrology<sup>2</sup>) has provided more precise explanations which help to clarify our thinking, and allow us to be more exact in what we are talking about.

### Defining 6 key terms

Six terms related to accuracy are: Precision, Trueness, Accuracy, Random Error, Systematic Error and Uncertainty. There is a logical relationship between these six terms, as shown in Figure 1 and Table 1.

#### Accuracy

is a combination of trueness and precision. Good accuracy requires good trueness and good precision. Accuracy is measured and reported as an uncertainty. Thanks to the growing popularity of laboratory requirements standards such as ISO 17025<sup>3</sup>, much has been written about uncertainty and the detailed procedures to how

to calculate or estimate uncertainty. Uncertainty is frequently presented in a very mathematical way, with lots of equations. The mathematical expressions in authoritative documents such as the guide to the expression of uncertainty in measurement<sup>4</sup>, can create the impression that uncertainty is a mysterious and difficult concept. It is helpful to remember that uncertainty is simply a quantitative expression that tells us the accuracy of a measurement.

### Precision

is a concept meaning that results are tightly clustered. Precision can be achieved regardless of where the cluster falls on the target. The degree of precision can be measured by quantifying the overall effect of all **random errors** using descriptive statistics such as standard deviation, relative

standard deviation (RSD) or coefficient of variation (CV). Precision is necessary for achieving good accuracy, but it is not sufficient. Good accuracy requires both precision and trueness.

### Trueness

is labeled on the vertical axis of Figure 1, and like precision is a concept. The measure of trueness is **systematic error**. The idea is that a measurement is true when it is aimed squarely at the center of the target. A pipette or automated liquid handler which is true, will deliver on average a result which is close to the center. As shown in the upper left target in Figure 1, it is possible to be true, while also having poor precision. Trueness and precision are independent of one another. Each can be increased or decreased without changing the other.

## For the mathematically inclined:

### To determine systematic error (%SE)

$$\bar{V} = \frac{1}{N} \sum_{i=1}^N V_i \quad \%SE = \frac{\bar{V} - V_T}{V_T} \times 100\%$$

$\bar{V}$  is the average of all measured volumes;

$N$  is the number of replicate deliveries;

$V_i$  is a single measured volume;

$V_T$  is the target volume, the volume intended to be delivered.

### To determine random error (%CV)

$$\%CV = \frac{100\%}{\bar{V}} \sqrt{\frac{\sum_{i=1}^N (V_i - \bar{V})^2}{N - 1}}$$

**Table 1. Relationships between error concepts and the way they are quantified.**

CONCEPT	QUANTITATIVE MEASUREMENT
Accuracy	Uncertainty
Precision	Random Error
Trueness	Systematic Error



### Q: When can a liquid handling device be considered reliable?

**A:** liquid handling device is reliable if it maintains accuracy over a period of time. The reliability can only be established by checking the method, using appropriate primary standards and control specimens<sup>3</sup>.

### Q: What is an outlier?

**A:** Outliers are extreme values which are outside of the expected range of values. They can occur by chance, but also may indicate an experimental error. Outliers should be investigated in an attempt to find a root cause. There are many different tests to identify outliers<sup>5</sup>.

### Q: How many data points should I collect when testing for precision and trueness?

**A:** The number of data points will vary depending upon your laboratory's needs, and whether you are testing for trueness, precision or both. Please see **Artel Lab Report, Issue 1, How Many Data Points**<sup>6</sup>.

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# Artel Lab Report

This publication provides technical information regarding the use, application, and metrology related to liquid handling instrumentation.

## Setting Tolerances for Pipettes in the Laboratory

### The performance of mechanical action pipettes

must be tested periodically to ensure accurate liquid delivery. The results of such testing then may be compared with pre-established tolerances, and out-of-tolerance conditions corrected. Tolerances that are too strict can cause a large number of so-called “false failures,” where a pipette in good working order produces test results that are out of tolerance. Tolerances that are too broad can degrade the quality of the laboratory’s analytical work.

This Lab Report reviews the key issues that should be considered when establishing tolerance limits for the working laboratory, and recommends a set of achievable tolerance limits for single and multi-channel pipettes of various sizes.

### Beware of manufacturers’ specifications

Users frequently find it difficult to reproduce a manufacturer’s performance claims, for a number of reasons:

- There are no consistent standards for how manufacturers set their performance claims. These claims are often a tradeoff between engineering judgment and marketing necessities.

- Pipette performance is influenced significantly by environmental factors such as temperature and humidity.<sup>1</sup> This means that a pipette calibrated at an environmentally controlled facility may deliver incorrectly on the benchtop.<sup>2</sup> Artel therefore recommends testing pipette performance under working conditions.
- The skill of the pipette operator plays a very important role in the accuracy, precision and trueness of the pipette.<sup>†</sup> The choice of pipetting technique (e.g., reverse mode versus forward mode) is also a source of variability in pipetting results.<sup>3</sup> Proper training can help reduce false failures by ensuring that results are valid and can be reproduced across operators. Artel’s guide, *10 Tips to Improve Your Pipetting Technique*<sup>4</sup>, is available for download at [www.artel-usa.com](http://www.artel-usa.com) to help you establish good pipetting practices in your laboratory.
- Pipette tips also affect testing results. Most manufacturers and reputable calibration services carefully specify the type of tips to be used when testing a particular pipette. If the user chooses another type of tip (e.g., a filter, elongated or gel loading tip), or a lower quality tip, the pipette can easily test outside of the manufacturer’s tolerances.
- Statistical factors such as the number of data points taken impact the probability of intermittent or false failures. For example, ten data points at each volume are recommended for testing to the tolerances in this Lab Report. Use of fewer than ten data points will increase uncertainty, and decrease the reliability of the test, which can be compensated for by tightening tolerances. Please see Lab Report 1 for a more complete discussion.

### Developing achievable tolerance limits

Based on our experience with many different makes and models of pipettes, including single and multi-channels, Artel recommends the values in Table 1 as a starting point for achievable tolerance limits. These limits are based on a simple guideline: Two percent of full scale at all volume settings.<sup>5</sup> For example, the systematic error for a 100  $\mu\text{L}$  variable-volume pipette is  $\pm 2.0 \mu\text{L}$  (2%) at the 100  $\mu\text{L}$  setting, and  $\pm 2.0 \mu\text{L}$  (4%) at the 50  $\mu\text{L}$  setting.

This type of generalized tolerance limit has been employed successfully in a number of other fields, such as humidity measurement and syringe calibration, where a fixed percentage of full scale reading is the customary means for specifying performance. The ISO 8655-2 standard for pipette testing<sup>1</sup> also uses a percentage of full scale approach.

“Fast is fine,  
but accuracy  
is final”

Kevin Costner  
as Wyatt Earp

<sup>†</sup> Definitions of accuracy, precision and trueness are detailed in Artel Lab Report 4

## Fine tuning tolerance limits

The tolerance limits recommended here are based on what is typically achievable by a reasonably skilled operator. These recommendations do not take into account the more stringent data quality requirements of a particularly demanding analytical method. In such circumstances, laboratories should evaluate the results of past testing to fine-tune the initial tolerance limits relative to the requirements of the method.

The following examples illustrate solutions for common problems encountered when establishing pipette tolerances.

**Example 1:** An analytical method requires dispensing a 100 µL sample with  $\pm 3\%$  systematic error. The laboratory has been using a 200 µL pipette set to 100 µL for this purpose. Table 1 shows the recommended tolerance to be 4%, which is too liberal for the method. The simplest and most reliable solution is to replace the 200 µL pipette with a 100 µL pipette. This pipette, when used at its full scale setting, can be tested against a 2% tolerance.

**Example 2:** An analytical method requires  $\pm 1\%$  systematic error at a volume of 1,000 µL. This is greater trueness than for any pipette in Table 1. Pipette performance data are examined to determine whether this degree of trueness can be attained. It is found that two particular operators are regularly attaining the desired level of performance when using a particular make and model of pipette, while other operators are not. The superior pipette is specified in the procedure, and the highly skilled operators are used as benchmarks against which others may be trained. The tolerance limit for this pipette can then be tightened to  $\pm 1\%$  without causing a large number of false failures.

### Using Table 1

Begin by choosing either the “Relative Error” or “Absolute Error” tolerance limit values. These tolerance limits reflect what is reasonably achievable in a working laboratory. They presume that the pipette is calibrated and functioning properly, is used with good quality tips, and is tested by a reasonably skilled operator. When these criteria are met, most makes and models of pipettes should test within these tolerance limits unless they are mechanically defective.

For fixed-volume pipettes, the nominal value is the fixed volume. For variable-volume pipettes, the nominal value is the largest user-selectable volume setting; e.g., a 10-100 µL pipette has a nominal volume of 100 µL.

The absolute error for the nominal volume applies to every selectable pipette volume; e.g., a 100 µL nominal volume yields limits of  $\pm 2.0$  µL systematic error (mean value) and less than 1.0 µL random error (measured as a standard deviation) for all volumes. The relative error varies throughout the pipette range; e.g., for a 10-100 µL pipette at 100 µL the relative systematic error is  $\pm 2.0\%$ . However, at 10 µL the relative systematic error is  $\pm 20.0\%$ .

**Table 1. Artel's suggested initial tolerance limits**

Pipette Volume, µL		Relative Error		Absolute Error	
Nominal	Setting	Systematic $\pm \%$ (Inaccuracy)	Random $\leq \%$ (CV)	Systematic $\pm \mu\text{L}$ (Inaccuracy)	Random $\leq \mu\text{L}$ (Std. Dev.)
2	2.0	2.0	1.0	0.04	0.02
	1.0	4.0	2.0		
	0.2	20.0	10.0		
2.5	2.5	2.0	1.0	0.05	0.025
	1.0	5.0	2.5		
	0.2	25.0	12.5		
10	10	2.0	1.0	0.20	0.10
	5	4.0	2.0		
	1	20.0	10.0		
20	20	2.0	1.0	0.4	0.2
	10	4.0	2.0		
	2	20.0	10.0		
50	50	2.0	1.0	1.0	0.5
	25	4.0	2.0		
	5	20.0	10.0		
100	100	2.0	1.0	2.0	1.0
	50	4.0	2.0		
	10	20.0	10.0		
200	200	2.0	1.0	4.0	2.0
	100	4.0	2.0		
	20	20.0	10.0		
500	500	2.0	1.0	10.0	5.0
	250	4.0	2.0		
	50	20.0	10.0		
1000	1000	2.0	1.0	20.0	10.0
	500	4.0	2.0		
	100	20.0	10.0		
2000	2000	2.0	1.0	40.0	20.0
	1000	4.0	2.0		
	200	20.0	10.0		
2500	2500	2.0	1.0	50.0	25.0
	1000	5.0	2.5		
	500	10.0	5.0		
5000	5000	2.0	1.0	100.0	50.0
	2500	4.0	2.0		
	500	20.0	10.0		

### Notes

**a)** Systematic error (sometimes referred to as inaccuracy) is expressed as the deviation of the mean of ten samples from the set point volume. Systematic error can be expressed in either absolute units such as microliters, or relative units such as percent. Random error is expressed in units of microliters as the standard deviation (Std. Dev.) of ten samples, or as the coefficient of variation (CV) of ten samples.

**b)** For single and multi-channel pipettes with nominal volumes between those provided in this table, systematic error limits are equal to  $\pm 2.0\%$  of the pipette's nominal volume, and the tolerance limit for random error is 1% of the pipette's nominal volume.

**c)** Relative error tolerance limits at other volume settings can be calculated by dividing the absolute tolerance limit (see Table 1 or Note b) by the set point volume. Multiply the result by 100 to convert it to a percentage.

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This publication provides technical information regarding the use, application, and metrology related to liquid handling instrumentation.

“...the key point is that the calibration schedule should be frequent enough to assure data validity...”

FDA, *Guidance for Industry Good Laboratory Practices Questions & Answers*<sup>1</sup>

## Calibration Frequency for Pipettes

### Understanding how pipettes fail

#### Silent Failures

Mechanical action pipettes, unlike the original glass pipette, contain many internal parts. Some pipette failures are evident, either to the eye or by the feel of the pipette action. In these instances, the operator is aware that the pipette is not operating correctly.

However, when the internal mechanism of a pipette fails, and it is not obvious to the operator, a silent failure has occurred. For example, a corroded piston or a leaking seal could cause the pipette to deliver incorrectly—sometimes by a wide margin—undetected by the operator.

#### Silent Failure Data

Figure 1 shows data taken at a major biomedical research institution. Fifty-three adjustable 2-20  $\mu\text{L}$  pipettes, then in service, were tested at 5  $\mu\text{L}$ . Each point on the chart

represents a pipette checked by a trained operator, using ten data points. Although all of the pipettes were in routine daily use, a number of them had failed and were performing outside the laboratory's established tolerances<sup>2</sup>. In all these cases, the operators were unaware that silent failures had occurred, and had not taken the pipettes out of service.

#### Random Failures

Pipette failure is considered random when it is due to accidents, misuse, or other unpredictable events. For example, an operator may accidentally draw liquid into the body of the pipette, causing piston corrosion or premature seal wear. In the real world of laboratory use, random failures cannot be prevented by infrequent scheduled maintenance.

As illustrated in Figure 2, random or unpredictable failures typically represent at least 90% of all pipette failures. In contrast, predict-

able (hence preventable) failures are those that arise from normal wear, which are dependent on factors such as frequency of use and time since last maintenance. Predictable failures represent 10% or less of all pipette failures.

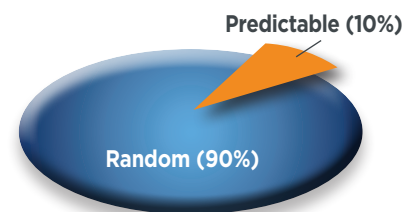


Figure 2. Pipette Failures in the Laboratory

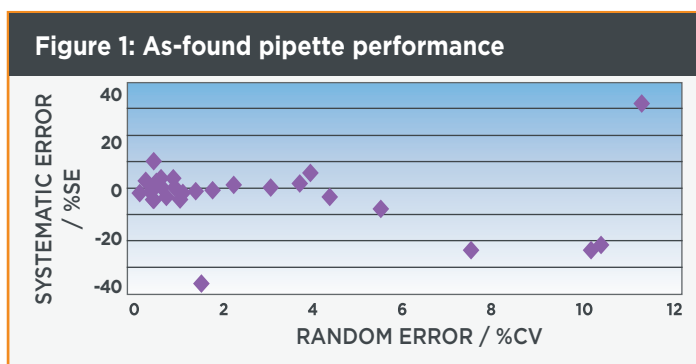
### Determining Calibration Frequency

#### Mean Time Between Failure

The average rate at which failures occur can be expressed as Mean Time Between Failure (MTBF). To determine MTBF, a group of pipettes is tracked to determine how long it takes each one to fail. A failure is defined as performance that falls outside the laboratory's established tolerances. The mean of all the failure times is the MTBF for that specific group of pipettes.

Once MTBF is determined, one can predict how long a pipette can be expected to maintain accuracy and precision.

MTBF, along with reliability level, QC principles, and regulations, combine to influence the development of a suitable calibration frequency. The MTBF for individual pipettes can vary significantly, depending on a number of factors, as shown in Figure 3.



## Conclusion

Whenever pipettes are used in a procedure, the corresponding laboratory results depend on the accuracy of pipette delivery. The quality control measures adopted for pipettes should therefore be consistent with quality control measures taken for other instruments in the laboratory.

Since pipettes are subject to silent and random failures, and have a higher rate of failure than most other laboratory equipment, the most important aspect of pipette quality control is a calibration frequency that achieves sufficiently high reliability. Calibration frequency is a function of the MTBF for the devices used in the lab, the lab's desired reliability level, and its established QC principles. Keep in mind also the important regulatory guidelines that pertain to your laboratory, to use as a foundation for establishing an appropriate calibration frequency.

Establishing  
an appropriate  
calibration  
frequency will  
minimize the  
chances that  
laboratory results  
are comprised by  
incorrect liquid  
delivery, helping to  
ensure traceability,  
accountability  
and confidence  
in the results.



### **Q: If I am running controls, why do I need to be concerned about checking my pipettes?**

**A:** Controls provide an important check on laboratory results. However, a control that falls within established limits does not provide a guarantee that all sample results are correct. For instance, if a pipette is failing intermittently due to leaking seals, then the controls may pass, yet some of the sample results would be incorrect due to pipette imprecision. A failed control tells you, at the end of the testing process, that something was wrong with the process, materials, or equipment. And, indeed, this was the way “quality” was achieved in many laboratories in past years. More recently, however, most laboratories have become convinced that it is both less expensive and more reliable to build quality into a laboratory result up-front than it is to discover the problems at the end of the process.

### **Q: How often do we need to perform preventive maintenance (cleaning, lubrication, seal replacement, etc.) on our pipettes?**

**A:** Manufacturers recommend maintenance anywhere from annually to every four years. While these recommendations provide a good starting point, maintenance schedules should be based on laboratory experience. The purpose of routine maintenance is to minimize the occurrence of predictable failures. A failed pipette should be examined to determine whether or not the failure was random (due to an accident or misuse), or predictable (the result of simple wear). Events that result in random failure will usually leave evidence; such as material aspirated into the pipette body, or damage to the shaft. Failures resulting from accumulated wear generally do not show these types of evidence. If a significant number of your failed pipettes do not show evidence of random failure, then you can assume such failures are due to wear, and you should consider increasing the maintenance frequency.

### **Q: If I perform regular preventive maintenance on my pipettes, do I need to worry about calibration?**

**A:** Yes. Pipette failures can happen silently at any time, at any point during your maintenance interval. Because failure can occur immediately after accidents or misuse, preventive maintenance cannot adequately protect against these random sources of failure. Note also that the random nature of most pipette failure in the everyday laboratory environment is not reflected in data from some pipette manufacturers. To obtain their data, these manufacturers subject their pipettes to a series of repetitive stress tests, carried out by laboratory robots under ideal conditions, resulting in predictable wear and gradual failures. Preventive maintenance can only prevent predictable failures. However, random (i.e., unpredictable) failures are best detected by the laboratory's established pipette calibration protocols. Effective calibration protocols, combined with appropriate preventive maintenance, comprise the best way to ensure accurate pipettes.

## Target Reliability Level

Another essential element in the determination of calibration frequency involves establishing a level of target reliability for liquid delivery, based on the quality mandate of the laboratory. Reliability level is expressed as a percent: 95% reliability means that, at any given time, 95% of the pipettes in a population are working correctly, while 5% are generating incorrect results.

Factors to consider when establishing a target reliability level include assay precision, the potential impacts of failed pipettes on patient outcomes, legal defensibility of results, production batch release decisions, and so forth. Compliance with regulatory guidelines may also be an important factor.

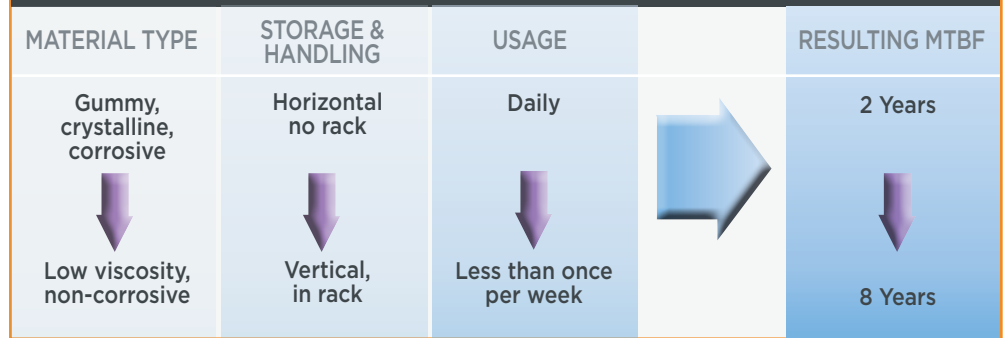
Given the established target reliability level for a laboratory and the MTBF for the pipettes, the graph in Figure 4 can be used to determine the required calibration frequency.

### Example:

Suppose that the required end of period reliability level for pipettes is 95% and the MTBF of the pipettes is four years.

To determine the appropriate calibration frequency, follow the green line of Figure 4 until it meets the 95% level on the Y-axis. Then read down to the X-axis to find the required calibration interval: slightly more than two months. Therefore, checking the pipettes at two-month intervals will provide assurance that pipette performance meets the established quality mandate.

**Figure 3. Factors contributing to Mean Time Between Failures (MTBF) for mechanical action pipettes**



## QC Principle

Mechanical action pipettes are precision laboratory instruments. For that reason, they should be subject to the same quality control principles as other sensitive instruments, such as spectrophotometers and balances. Just as is required for these instruments, calibration should be performed on a regular basis to verify pipette performance.

The more frequently calibration is performed, the sooner malfunctioning pipettes will be detected and taken out of service. In addition, more frequent calibration can help eliminate the need to review laboratory data to ensure that incorrect liquid delivery by a failed pipette has not compromised laboratory results. The longer a defective pipette remains in service, the greater the liability it presents in this regard.

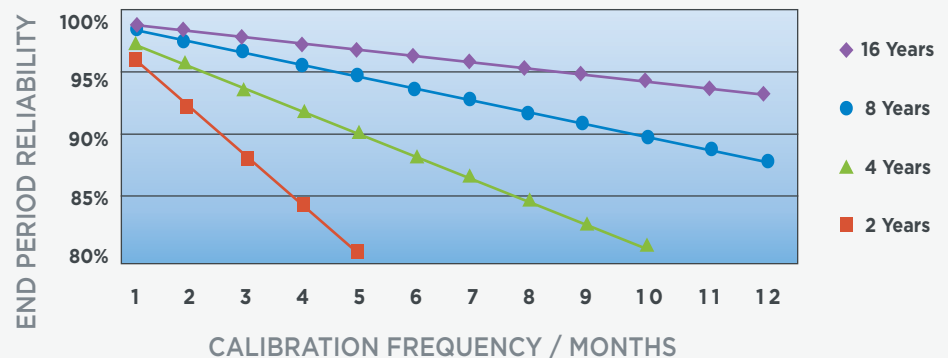
## Regulations

In order to build quality into laboratory results, the instruments used in the process must be in good condition and properly calibrated. Regulations and standards published by organizations such as the U.S. Food and Drug Administration (FDA)<sup>1</sup>, and ASTM<sup>6</sup> International provide minimum requirements that help ensure the quality of laboratory results. These form the groundwork upon which a laboratory should establish its frequency of pipette calibration, as part of good quality control practices.

Regulations specify that all laboratory instruments used in production—pipettes included—must be routinely calibrated at suitable intervals. In particular, FDA GLP<sup>3</sup>, GMP<sup>4</sup>, and QSR<sup>5</sup> requires that control of measurement test equipment include procedures for establishing calibration intervals.

The Clinical and Laboratory Standards Institute (CLSI) recommends that pipettes (single and multi-channel) and automated liquid handlers be calibrated every 3 to 6 months. A minimum of two volumes must be tested (nominal and lowest setting) with ten replicates at each volume. An additional test at 50% of nominal volume is recommended<sup>7</sup>. Therefore, both the MTBF described in this article and applicable regulations and guidelines should be considered.

**Figure 4. Calibration frequency for pipettes, based on target reliability level and estimated Mean Time Between Failures (MTBF)**





**Q: I use the “tip drip test,” aspirating liquid into my pipette tip and observing whether any liquid drips out. Is this as sufficient a check as calibration?**

**A:** In a high-volume pipette, the “tip drip test” will sometimes indicate a seriously leaking seal. Unfortunately, with low-volume pipettes, surface forces prevent liquid from dripping out of the pipette tip, even when seal leakage is very severe. And even with high-volume pipettes, a tip drip test may not uncover other problems, such as intermittent leakage, or leakage during only one part of the pipetting cycle. These types of failures are best detected during calibration.

**Q: Do the same checking guidelines apply for multi-channel pipettes as for air displacement, single-channel pipettes?**

**A:** The same guidelines do apply. For a multi-channel pipette, it is important to check the function of each channel, since they can develop problems independently. A practical procedure would be to verify one channel, using ten data points, at each of three different volume settings. Then verify that the other channels are performing properly, by using fewer data points at two volume settings.

**Q: Our pipettes undergo a vacuum test after maintenance or repair. Does that mean we don't need to calibrate them?**

**A:** No. Calibration is still essential to ensure correct pipette operation. A vacuum test can only detect air leaks. While it can usually determine whether new seals and o-rings have been installed correctly, a vacuum test tells you nothing about whether the pipette is correctly adjusted to deliver the proper volume. A further concern is that vacuum testing frequently cannot detect small leaks; it is therefore not suitable even as a leak test for low-volume pipettes. Additionally, in regulated environments, guidelines mandate full performance verification before reintroducing a device into service. This explicitly renders vacuum testing an inadequate substitute for pipette calibration. In short, vacuum testing is no bill of good pipette health where accuracy and precision are concerned.

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# Artel Lab Report

This publication provides technical information regarding the use, application, and metrology related to liquid handling instrumentation.

“The difference between failure and success is doing a thing nearly right and doing it exactly right.”

—Edward Simmons

## Facilitating **Assay Transfer** by Controlling Liquid Handling Variables

Assay transfer is one of the most complex activities in laboratories today.

Successful assay transfer ensures that new results are comparable to historical data. Whether it involves scaling an assay up to a higher throughput platform or transferring an assay from development to a QC environment, assay transfer is challenging because of the large number of variables involved. Differences in equipment, reagents, technique and interpretation of methodology are typically the focus of preparation before assay transfer. Less obvious variables, such as those that involve liquid handling, are often the cause of assay transfer failures. These frequently ignored variables are difficult to identify and, therefore, to troubleshoot.

Liquid handling instruments, such as pipettes and automated liquid handlers, are routinely used to perform assays in laboratories. Their accuracy is often taken for granted and, therefore, not given proper attention during assay transfer work. Understanding the importance that liquid handling plays in assay transfer work and taking measures to control liquid handling variables will ultimately facilitate assay transfer.

For example, errors in liquid delivery can often be unnoticed, undocumented, or misunderstood. These errors may come from one or more sources, such as pipettes, liquid handlers, operators, environmental conditions, and labware.<sup>1-4</sup> Liquid handling errors propagate and can significantly impact the assay. These errors result in the time-consuming challenges of diagnosing and troubleshooting the problem, hence jeopardizing the process before the assay is successfully transferred.

Successful assay transfers are a product of careful planning and advance preparation. Preventing liquid handling problems during execution involves pre-transfer work, such as understanding the assay, focusing on training and calibration, and generating effective documentation.

**Table 1: Parameters that affect a working assay**

### Assay Parameters

Plate or tube format (96, 384, etc.)  
Selected analyte concentration  
Number and type of dilution steps  
Number of liquid transfers  
Number of incubation or centrifugation steps  
Number of wash steps  
Dispense order  
Liquid properties (viscosity, temperature, etc.)

### Instrument Parameters

Target or off-set volume  
Single vs. multi-sequential dispenses  
Reagent mixing protocol  
Aspirate/dispense rate  
Aspirate/dispense height  
Pertinent liquid class settings  
Accuracy of volume transfer  
Overall speed between transfers (time delays)  
Precision of volume transfer  
Wet vs. dry dispense  
Type of liquid handler/pipettor:  
• Number of tips  
• Air displacement, system fluid, acoustic ejection  
Types of Tips:  
• Max/min volume capacity  
• Fixed vs. disposable  
• Dry tip vs. wet tip  
• New tip vs. used tip  
• Carryover  
• Tip touches  
• Sterilized vs. unsterilized disposable tips  
• Use of filter or specialty tips  
• Tip quality

### Laboratory Parameters

Operator skill and technique standardization  
Environment, temperature, and humidity

**Figure 1: Pre-training skills assessment**



Figure 1. Pipetting skills assessment of fifty-three QC technologists prior to receiving pipetting technique training, dispensing 10  $\mu$ L aliquots of sample (n=5) using the same calibrated pipette.

**Figure 2: Post-training skills assessment**

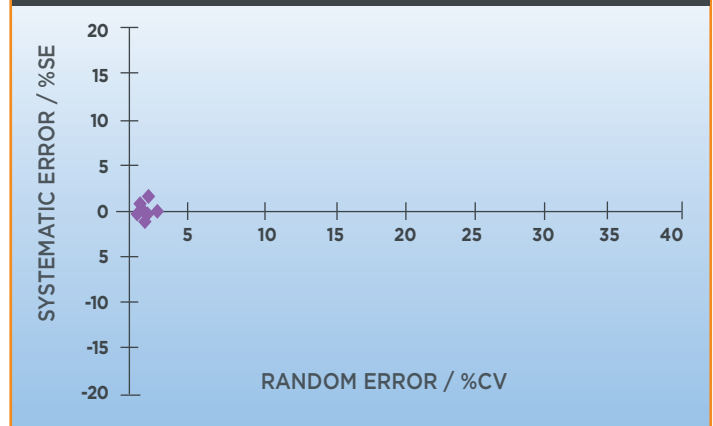


Figure 2. Post-training data for the same technologists, performing the same skills assessment protocol, with the same pipette, as described for Figure 1.

## Post-Transfer Activities

### Sustaining Knowledge Transfer

A complete, successful assay transfer is the foundation for ensuring that comparable, high quality results are generated at the receiving site. Sustaining the knowledge obtained during the execution of a method transfer in a new site involves robust SOPs, method maintenance and, as mentioned in Pre-Transfer Activity #2, strong training and calibration programs.

Proper and detailed documentation captures method nuances discovered during the transfer exercise. SOPs are typically refined after the assay is transferred to clarify and expand on the instructions provided in the original document. Furthermore, assay controls implemented as part of the transfer process must be added to the SOP to guarantee the assay is executed correctly.

Maintaining a transferred method can be accomplished in many ways, depending on the purpose of the assay. Comparability studies, trending assessments and regular revalidation schedules are typically put in place to ensure that the transferred assay continues to perform as expected.

## Conclusion

Liquid handling steps are a controllable but often ignored variable in assay transfers. Understanding the impact of volume delivery on the assay, implementing appropriate pipette and liquid handler calibration and training programs, and developing effective documentation that addresses the details of liquid handling steps are pre-transfer preparation activities that are essential to the success of an assay transfer. They allow the transferring and receiving sites to approach the assay transfer with confidence by eliminating variables and, therefore, improving the chances for success. Once an assay is successfully transferred, these strategies are still applicable to sustaining the assay at the new site by, for example, keeping the equipment functioning properly and capturing method details in SOPs.

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Preventing liquid handling problems during execution involves pre-transfer work, such as understanding the assay, focusing on training and calibration, and generating effective documentation.

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**Table 2. Common methods used to evaluate volumetric performance**

Method	Description
Gravimetry	The liquid is dispensed into a container and the container is weighed on an analytical balance; the liquid density value is used to calculate volume transferred by the pipette.
Absorbance	A dye-based solution is dispensed with the liquid handler and, depending on the extent of the methodology, the absorbance data are correlated to compute volume.
Fluorescence	A fluorescent dye-based solution is dispensed with the liquid handler and, depending on the extent of the methodology, the fluorescence data are correlated to the volumetric precision.
Dual-dye Photometry	Dual-dye, dual-wavelength ratiometric photometry is used to assess accuracy and precision of volume transfers in one measurement.
Combination of Methods	Combining some of the above methodologies helps achieve precision and accuracy information. For instance, an absorbance method to determine precision can be combined with a gravimetric method to determine trueness.

### Pre-Transfer Activity #1 Understand the Assay

Understanding the liquid handling steps in the assay is important because variable liquid delivery will alter analyte and critical reagent concentrations, which in turn affect the accuracy of the assay.

Begin by identifying the critical liquid handling steps in the assay. To accomplish this, list all the liquid handling steps performed and describe their execution in detail, drawing on the parameters compiled in Table 1.<sup>4</sup> Using this detailed list, evaluate the risk at each liquid handling step, and pinpoint the areas that could be problematic during transfer.

Next, evaluate the accuracy of the volume delivery that will occur at the critical steps between the assay transfer sites.<sup>5</sup> Table 2 lists the common methods for evaluating volumetric performance. Implementing a volume verification process ensures that the performance of liquid handling instrumentation is known, which allows laboratories to achieve the same relative analyte and reagent concentrations in the assay at both the transferring and receiving sites.

### Pre-Transfer Activity #2 Implement Calibration and Training Programs

Pipettes and liquid handlers require calibration and preventive maintenance schedules that ensure the accuracy, precision and trueness of each volume dispensed under normal operating conditions. Properly maintained and calibrated volume delivery instrumentation at the transferring and receiving sites is an easy-to-control variable that minimizes assay transfer failures. Calibrating to a known standard available at each site will further reduce the potential for failures.

Further, technologists pipetting skills can be a relatively large unknown source of error<sup>2</sup> encountered during assay transfer. Operator error may go undetected, resulting in poor assay performance and failed assay transfers. Figures 1 and 2 illustrate the differences in pipetting skills in terms of precision (%CV) and trueness (%SE), before and after training for fifty-three QC technologists. A significant improvement in pipetting skill was observed post-training. Hence, a comprehensive training program is necessary to control the variable of technologist pipetting skills, and to standardize pipetting technique, prior to assay transfer.

### Pre-Transfer Activity #3 Develop Effective Documentation

Generating effective documentation is another critical part of pre-transfer work. A detailed standard operating procedure (SOP) describing the assay, as it is executed in the transferring site, is key because it provides a standard for training and executing at the receiving site.

Robust and detailed SOPs created during or after assay development at the transferring site provide information that can further define assay steps to reduce problems at the receiving site. For instance, detailing the liquid handling steps in Pre-Transfer Activity #1 will allow the receiving site to focus on details that may have otherwise been overlooked, and will ultimately lead to a successful transfer.

Another important documentation component is the transfer protocol. A formal protocol defines the expectations for the transfer exercise, provides agreement between the sites on how the transfer will be executed, and defines the criteria for a successful assay transfer. A comparison between the results for identical samples at both sites is required, and an equal number of determinations for each sample at each site is advisable to simplify statistical analysis. Analytical precision studies including repeatability, intermediate precision and inter-laboratory reproducibility are recommended.<sup>6</sup> Poor precision in liquid handling can have a direct effect on repeatability, while poor liquid handling accuracy would contribute to poor reproducibility. Finally, analytical accuracy is evaluated by determination of the percent difference for results for identical samples at both sites using the results of the transferring site as the “true value.”<sup>6</sup> So, it is important that liquid handling accuracy be consistent at both laboratories.



**Q: What are some common operator pipetting technique errors?**

**A:** Several pipetting actions can contribute error to a laboratory process. Failing to pre-wet the pipette tip can lead to inaccurate liquid delivery by delivering lower volumes than expected. Excessive tip wiping can also cause sample absorption and under-delivery. Choosing the wrong pipetting mode is another common error. For instance, reverse mode pipetting can be more ideal for viscous samples, whereby the plunger is depressed past the first stop to aspirate the aliquot from the sample, and depressed only to the first stop to deliver the aliquot. Finally, variability in the temperature of the liquid significantly contributes to error in pipetting. For more information, see Reference 2.

**Q: Will the disposable tips on automated liquid handlers contribute error during assay transfer?**

**A:** When using disposable tips, the tip quality and characteristics are critical to the integrity of the volume transfer. Vendor-approved tips, as opposed to the less expensive 'bag of tips' option, should always be employed to minimize volume transfer error and optimize liquid delivery quality. Disposable tip performance has been found to be directly related to quality because tip material, shape, properties, fit and wet-ability are all important factors for repeatability. The cheaper, bulk tips may not be manufactured with the highest precision manufacturing and may have variable characteristics that affect delivery, such as differences in upper diameter, virgin plastic content and presence of plastic residue inside the tip, known as "flash." These tips also might not fit well on the liquid handler and they may have variable wetting/delivery properties. Without using approved tip types, accuracy may be at risk. See Reference 1 for more information.

**Q: Can fixed tips on liquid handlers contribute error during assay transfer?**

**A:** Fixed tips are usually stainless steel pipetting channels which are used repeatedly, often with wash steps between pipetting steps. These tip types may also present sources of variability in liquid delivery with automated liquid handlers. Continued use of fixed tips with caustic reagents may degrade the inside surface of the tips and create grooves or pockets in the tip material. Liquid may fill the formed cavities during volume transfers resulting in carry over, contamination or even over-delivery of solution to critical assays. Additionally, many liquid handlers that use fixed tips require system fluid to aspirate and dispense the sample volume (and the system fluid aids with the washing steps between pipetting events). Error can creep into a process when insufficient air gaps are employed between the system fluid and sample volume, which results in a diluted sample, i.e., less concentrated sample volume and cross-contaminated system fluid. For more information on identifying sample dilution from system fluid as well as circumventing such issues, see References 7 and 8.

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