Artel Lab Report 5

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

Setting Tolerances for Pipettes in the Laboratory

"Fast is fine, but accuracy is final"

Kevin Costner
as Wyatt Earp

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.[1] This means that a pipette calibrated at an environmentally controlled facility may deliver incorrectly on the benchtop.[2] 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.[†] The choice of pipetting technique (e.g., reverse mode versus forward mode) is also a source of variability in pipetting results.[3] 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***[4], is available for download at 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.[5] For example, the systematic error for a 100 µL variable-volume pipette is ±2.0 µL (2%) at the 100 µL setting, and ±2.0 µL (4%) at the 50 µL 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[1] also uses a percentage of full scale approach.

† Definitions of accuracy, precision and trueness are detailed in Artel Lab Report 4

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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 ±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 ±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 ±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 ±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 ±2.0%. However, at 10 µL the relative systematic error is ±20.0%.

Table 1. Artel's suggested initial tolerance limits

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

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 ±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.

References:

1. Piston-Operated Volumetric Apparatus—Part 2: Piston Pipettes. ISO 8655-2:2002.
2. Bias, Uncertainty and Transferability in Standard Methods of Pipette Calibration. Artel white paper, 2003.
3. Carle, et. al. *Best Practices for the Use of Micropipets* **www.artel-usa.com/resource-library/best-practices-use-micropipets/**
4. 10 Tips To Improve Your Pipetting Technique: **www.artel-usa.com/resource-library/10-tips-to-improve-your-pipetting-technique/**
5. Clinical and Laboratory Standards Institute, General Laboratory Equipment Performance Qualification, Use and Maintenance, 2019 : clsi.org/standards/products/quality-management-systems/documents/qms23/

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