ARTEL® lab report
Issue 5

Setting Tolerances

for pipettes in the laboratory

"Fast is fine, but accuracy is everything"

Wyatt Earp

The performance of mechanical action pipettes must be tested periodically to ensure accurate and precise 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.

Further complicating the situation is the fact that pipette performance is dependent upon factors beyond the mechanical condition of the pipette. Balancing this interplay of factors can make it a challenge to develop achievable tolerance limits for pipettes. This Lab Report reviews the key issues that should be considered when establishing tolerance limits for the working laboratory, and recommends as a starting point a set of achievable tolerance limits for pipettes of various sizes.

Beware Manufacturers' Specifications

Many laboratories attempt to apply pipette manufacturers' specifications as the tolerance limits for their in-lab testing program. However, a manufacturer's specifications may not accurately reflect the performance attained in the working laboratory.

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 trade-off 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.[3]
- The skill of the pipette operator plays a very important role in the precision and accuracy of the pipette.[4] The choice of pipetting technique (e.g., reverse mode versus forward mode) is also a source of variability in pipetting results. 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,* is available on request to help you establish good pipetting practices in your laboratory.
- The type of pipette tip used in the testing also affects results.[1, 5] Most manufacturers and reputable calibration services precisely define the type of tips to be used when testing a particular pipette. If the user chooses another type of tip (e.g., a filter 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, four data points are sufficient for a "quick check" on accuracy and proper operation[5], but ten data points are recommended to ensure both accuracy and precision.

Developing Achievable Tolerance Limits

Based on our experience with many different makes and models of pipettes, Artel recommends the values in Table 1 as a starting point for developing achievable tolerance limits. These limits are based on a simple guideline: *Two percent of full scale at all volume settings.*[6] For example, the inaccuracy tolerance 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 conformity testing[1] also uses a percentage of full scale approach.

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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 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 inaccuracy (mean value) and ±1.0 imprecision (STD) for all volumes. The relative error varies throughout the pipette range; e.g., for a 10-100 µL pipette at 100 µL the relative inaccuracy is ±2.0%. However, at 10 µL the relative inaccuracy is ±20.0%.

Notes

a) Inaccuracy is expressed as the deviation of the mean of ten samples from the set point volume. Inaccuracy can be expressed in either absolute units such as microliters, or relative units such as percent. Absolute imprecision is expressed as the standard deviation (STD) of ten samples. Relative imprecision is expressed as the coefficient of variation (CV) of ten samples.

b) For pipettes with nominal volumes between those provided in this table, absolute inaccuracy tolerance limits are equal to ±2.0% of the pipette's nominal volume and the tolerance limit for absolute imprecision 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.

Table 1: Artel's suggested initial tolerance limits

Pipette Volume, µL		Relative Error		Absolute Error	
Nominal	Setting	Inaccuracy ± %	CV %	Inaccuracy ± µL	STD µL
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

The allowable percentage that ISO specifies is fairly constant for medium- to large-volume pipettes, but increases for the smallest volume pipettes. This deviation is necessary when using gravimetric methods in order to accommodate the increased error inherent in gravimetry at smaller volumes.[7] If a more exact method such as ratiometric photometry is used, then a constant percentage of full scale may be applied to even the smallest volume pipettes.

Fine-Tuning Tolerance Limits

The tolerance limits recommended here are based on what is typically achievable when testing pipettes in the absence of rigorous laboratory environmental controls or specialized training in pipetting technique. 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% accuracy. 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% accuracy at a volume of 1,000 µL. This is a greater accuracy than given for any pipette in Table 1. Pipette performance data are examined to determine whether this degree of accuracy 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.

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. Rodrigues, George. "Put That in Your Pipette…and Calibrate It," *Pharmaceutical Formulation and Quality,* February/March, 2003: 64-65.

4. Pentheny, G. "Effects of common pipette technique errors on accuracy and precision," *American Clinical Laboratory* (1997), 16(2): 10-12.

5. *Standard Specification for Piston or Plunger Operated Volumetric Apparatus,* Specification E 1154-89 (Re-approved 2003), American Society for Testing Materials (ASTM), p. 5, ¶ 11.2.2 and 11.2.3.

6. Duff, L. David. Metrology A-Z, Inc., personal communication, 2003.

7. Clark. John P. and Shull, A. Harper. "Gravimetric & Spectrophotometric Errors Impact on Pipette Calibration Certainty." *Cal. Lab.*, 10 (1): pp. 36-43, 2003.

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