# Artel Lab Report 1

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[1] ISO[2], and CLSI[3] 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 systemactic error ($SE_U$) and percent precision ($CV_U$). These are limits that you would expect the pipette to exceed only rarely.

### Know your limits.

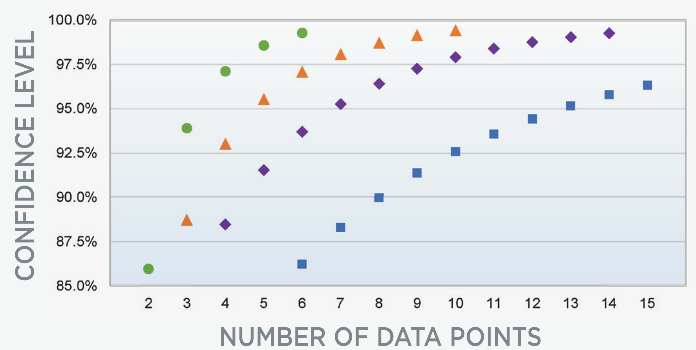
It is very important that your test procedure for trueness and precision delivers results well within your upper limits. Choose test limits ($SE_T$ and $CV_T$) which you can meet consistently, given a reliable pipette.

### 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**




*USE CRITICAL VALUES FOR TRUENESS (CVSE )*

ARTEL
trust your results

## 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

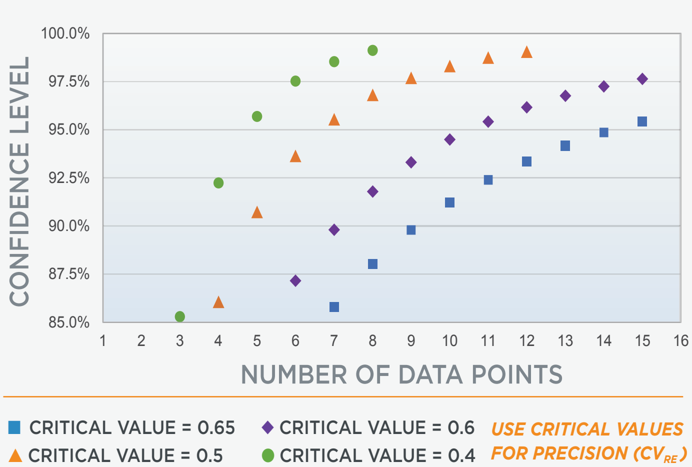
Suppose that your most demanding applications cannot tolerate systematic error greater than 3% ($SE_U$) or random error greater than 2% ($RE_U$). Based on your history of pipette testing data you are reliably able to achieve systematic error below 2% ($SE_T$) and random error below 1% ($RE_T$) when testing 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**




# Q&A

**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%[6], 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[4,5]. For the precision calculation, it is assumed that the Upper Limit is based on a test using 30 data points.*

### References:


1. ASTM E 1154-14 *Standard Specifi cation for Piston or Plunger Operated Volumetric Apparatus.*
2. ISO 8655-2: 2002 Piston-operated volumetric apparatus —Part 2: Piston pipettes
3. *Clinical and Laboratory Standards Institute, General Laboratory Equipment Performance Qualifi cation, Use and Maintenance, 2019 : clsi.org/standards/products/quality-manage ment-systems/documents/qms23/*
4. Mandel, John. *The Statistical Analysis of Experimental Data, General Publishing Co, 1964.*
5. *Snedecor GW, Cochran WG. Statistical Methods, Iowa State University Press, 1989.*
6. Carle, et. al. *Best Practices for the Use of Micropipets* www.artel.co/resource-library/best-practices-use-micropipets/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