ARTEL® lab report
Issue 1

How many data points?

> "It is a deplorable misconception, based on ignorance or disregard of elementary statistical facts, that small [numbers of] samples are sufficient to obtain satisfactory estimates of variability."
>
> John Mandel,
> *The Statistical Analysis of Experimental Data*

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 or 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 correctly? Let's step through a process which can help you evaluate your laboratory's needs.

What are the regulatory guidelines?

Several regulatory agencies, including ASTM[1], NCCLS[4], and ISO[2], specify taking four data points monthly for a quick check, and ten data points quarterly for a comprehensive check. This recommendation is generalized and does not take into account the needs of individual laboratories.

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 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 checking procedure around it. For this application, determine Upper Limits for percent inaccuracy (E_U) and percent imprecision (CV_U). These are limits that you would expect the pipette to exceed only rarely.

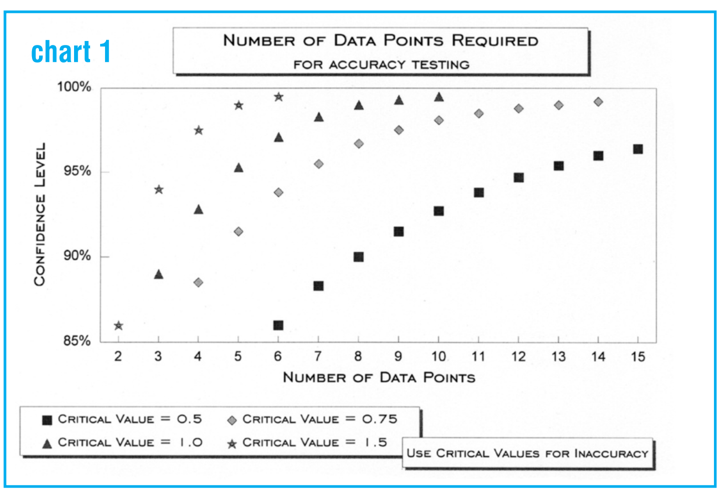
Know your Limits.

It is very important that your test for accuracy and precision delivers results well within your Upper Limits. Choose Test Limits (E_T and CV_T) which you can meet consistently, given a reliable pipette.

Establish critical values using the following formulas:

Critical Value for Inaccuracy= $(E_U-E_T)/CV_T$

Critical Value for Imprecision= CV_T/CV_U



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Establish a Confidence Level for your testing.

This is the probability of checking and passing a pipette and putting it back into service with the certainty that the pipette did not pass because of statistical chance. Many laboratories require a Confidence Level of 95%.

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

The number of data points for inaccuracy testing may be different from that required for imprecision. 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 inaccuracy greater than 4%(E_U) or imprecision greater than 2.5%(CV_U). You are reliably able to achieve inaccuracy below 2%(E_T) and imprecision below 1.5%(CV_T) when testing good pipettes, so these are the limits you impose on all pipettes tested. You require a Confidence Level of 95%.

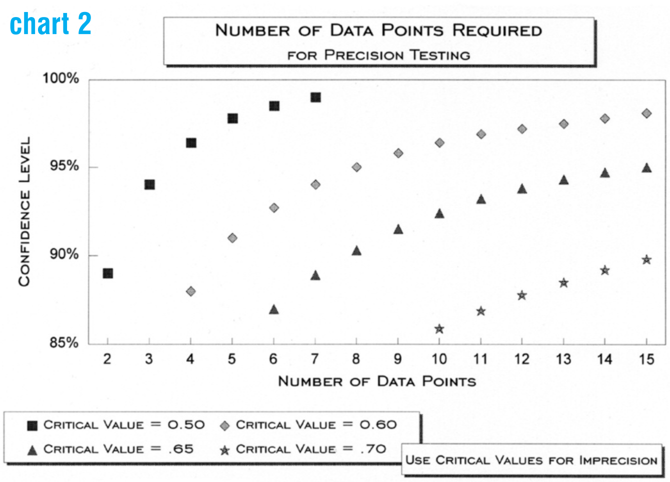
Calculate the Critical Value for Inaccuracy = (4% - 2%) / 1.5% = 1.33. Determine the number of data points required from Chart 1 by interpolating between the (critical value = 1.0) and (critical value = 1.5) curves at the 95% confidence level. You find that 4 data points are needed to satisfy the accuracy testing requirement. By taking 4 data points and maintaining an inaccuracy of lower than 2%, you establish with a 95% confidence level that the pipette, given a stringent test, would not fall outside the 4% upper limit of inaccuracy.

In a similar way, the Critical Value for Imprecision can be computed (1.5% / 2.5% = 0.60) and the number of data points required read from Chart 2 (9 data points at the 95% confidence level).

Overall, you need to take at least 9 data points to satisfy both the inaccuracy and imprecision requirements of the test.

The charts are based on the same statistical methods which are used and presented in National Committee for Clinical Laboratory Standards (NCCLS)[4], American Society for Testing and Materials (ASTM)[1], and International Organization for Standardization (ISO)[2] documents on pipette calibration. If you wish to calculate Critical Values other than those shown on the charts, use $+t/\sqrt{n}$ for accuracy 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 any statistics reference[3,6]. For the imprecision calculation, it is assumed that the Upper Limit is based on a test using 30 data points.

chart 2



Questions & Answers

Who should check our pipettes?

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%[5], 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.

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

No, not necessarily. While it is possible for an accurate 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 stringently, perhaps with more data points.

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?

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 good, solid, objective, 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.

References:

1. ASTM E1154 *Standard Specification for Piston or Plunger Operated Volumetric Apparatus*.
2. ISO DIS 8655/3 *Piston and/or Plunger Operated Volumetric Apparatus* (POVA) *Part 3: Methods of Test*. (draft)
3. Mandel, John. *The Statistical Analysis of Experimental Data*, General Publishing Company, 1964.
4. NCCLS I8-P Determining Performance of Volumetric Equipment. (draft)
5. Pentheny, Gail. "Effects of Common Pipetting Technique Errors on Accuracy and Precision," *ACL* March1997.
6. Snedecor GW, Cochran WG. *Statistical Methods*, Iowa State University Press, 1989.

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