Forensic Science Communications

October 2003 – Volume 5 – Number 4

Research and Technology

Table of Contents
Back Issues
Search

Editors
About FSC
Submissions

Eliminating Sources of Pipetting Error in the Forensic Laboratory

David M. Epstein
Director of Scientific Operations

Ian R. Tebbett
Director of Training and Education

Shannon E. Boyd
Laboratory Support Analyst
National Forensic Science Technology Center
Largo, Florida

Abstract | Introduction | How Pipettes Work | Impact of Operator Technique
Reducing Mechanical Pipette Error | Calibration Guidelines | Conclusion
Ten Tips for Improving Pipetting Technique | References

Abstract

Air-displacement pipettes are used to perform so many analytical methods that they are often taken for granted. Pipettes are complex precision instruments subject to error due to mechanical failure and improper operator technique. Pipettes may contribute more inaccuracy and imprecision to laboratory results than any other single source (Conners and Curtis 1999). In the forensic laboratory, where data integrity must be above reproach, it is vital for criminalists and managers to understand and address the likely sources of error related to pipette function and technique.

Introduction

The mission of the National Forensic Science Technology Center (located in Largo, Florida) is to assist the forensic sciences to achieve the highest level of quality services for the justice system. The mission is accomplished by providing training, education, and quality systems support. One key element that impacts the accuracy and precision of countless laboratory procedures is pipette performance. Pipettes and pipetting technique are often taken for granted which is a serious oversight that can cost far more than time and money when criminal cases are concerned.

This paper will discuss potential sources of pipetting error in the forensic laboratory and offer suggestions for mitigating these sources of error by straightforward quality control and operator-training guidelines.

How Pipettes Work

The calibrated-glass pipette has helped form many opinions about today's mechanical pipetting devices. Glass pipettes have no moving parts. They have two operational states—fully functional or broken. If a glass pipette is cracked or chipped, or a bubble is drawn into it, the problem is readily evident to the user. In addition, accuracy and precision are largely independent of technique.

The modern air-displacement pipette, in contrast, is a precision instrument. It is highly sensitive to environmental conditions and has many moving parts that are affected by wear, accidents, or misuse (Curtis 1994, Part 1). As shown in Figure 1, there are many points of contact between air and liquid in a modern pipette (e.g., between the shaft and its seals, the piston and its seat, the nosecone and the tip). Faulty sealing in one or more of these interfaces is the most common cause of pipette failure. Furthermore, any of the pipette's small internal parts are subject to various forms of damage, from corrosion to contamination to under- or overlubrication, which can skew the results obtained with it. Operational failures that result from these types of wear and damage are rarely noticeable to the user; thus, they are termed silent failures (Curtis 1994, Part 2).

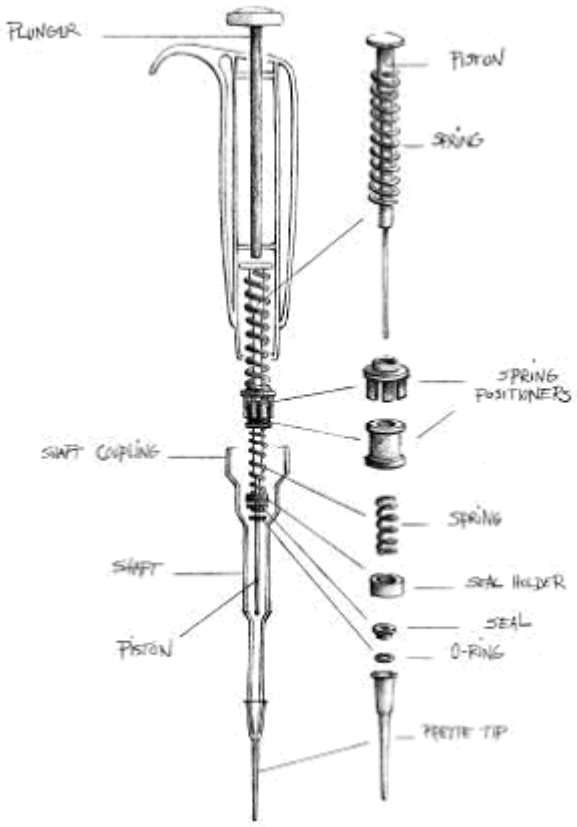

Figure 1: Parts of an Air-Displacement Pipette (Reproduced with permission from Artel, Incorporated)

One reason pipettes fail is that they are used for a wide range of procedures. If pipettes were exercised by robotic equipment, their rate of failure could be systematized based on wear. Under most laboratory conditions, however, the human factor comes into play. Pipettes may be scratched, dropped, and contaminated by corrosive liquids. The everyday abuse that these devices take is the key reason why pipette failure is so difficult to predict (Curtis 2000). This problem is exacerbated by the widely held assumption that the operator can ascertain whether a pipette is performing properly. Unfortunately, this is rarely the case. Consequently, laboratories whose calibration programs are inadequate risk repeated undetected instances of random failure.

Figure 2 illustrates how a pipette's design and state of repair can affect its

functioning. It also serves as an example of what is meant by the terms accuracy and precision; terms that are often used to describe pipette performance. This figure presents a summary of calibration data for four pipettes. The data were collected in a single testing session by a properly trained operator using the Artel PCS® Pipette Calibration System (Artel, Incorporated, Westbrook, Maine, www.artel-usa.com), a photometric device that uses a colorimetric method to determine the volume delivered by a pipette. The liquid dispensed was Artel PCS® Sample Solution (Artel, Incorporated, Westbrook, Maine, www.artel-usa.com), which is engineered to perform like degassed distilled water in an air-displacement pipette. The calibrations were performed under environmental conditions recommended by the manufacturer.

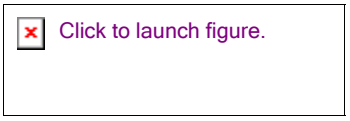

Figure 2: Variability in Pipette Accuracy and Precision

- Pipette A is a 10-100µL variable-volume pipette set to deliver 100 percent of its maximum volume. It is accurate and precise.
- Pipette B is a 100-1000µL variable-volume pipette set to deliver only 10 percent of its maximum volume, or 100µL. Degraded precision is seen even though it delivers close to the 100µL target volume. This is to be expected given that pipettes generally become increasingly less precise when used at the lower range of their maximum volume, as reflected in manufacturers' specifications and applicable international standards (ISO 2002).
- Pipette C is a 10-100µL variable-volume pipette set to deliver 100µL. It is most likely misadjusted. It is precise (repeatable) but delivers about 105µL (5 percent high) and is, therefore, inaccurate.
- Pipette D is another 10-100µL variable-volume pipette set to deliver 100µL. It is about to fail or already has. It is neither accurate nor precise. This pipette is underdelivering in the first four dispenses, most likely due to a leaky o-ring; then it seems to reseal itself temporarily. The pipette needs to be repaired. This pattern of random, undetectable, and unpredictable failure is typical of many instances of seal failure.

Impact of Operator Technique

At one of the first National Forensic Science Technology Center's training sessions, variability in pipetting technique was evaluated. Figures 3 and 4 summarize the results of a study that took place at the Center in March 2002.

Prior to the training, 11 criminalists were evaluated for pipetting-technique competency. All were familiar with laboratory procedures, including the use of air-displacement pipettes. Each student was asked to pipette 10µL using a known, good pipette that had been recently calibrated. The pipettes used were 0.2-20µL variable-volume pipettes set at 50 percent of maximum volume, or 10µL. Ten data points were obtained from each student, and the data was analyzed for precision and accuracy using the Artel PCS® Pipette Calibration System. The data in Figure 3 shows a number of these laboratory professionals exhibited significant variability in pipetting competency.

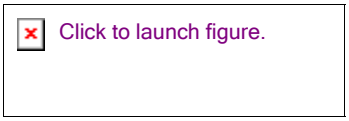

Figure 3: Variability in Pipetting Competency Prior to Training

Following the initial collection of pipetting data, representatives from Artel, Incorporated, trained the same students in proper pipetting technique. The training consisted of a brief discussion, followed by ten minutes of one-on-one instruction. Using the Artel PCS® Pipette Calibration System, participants were able to practice pipetting technique, while receiving immediate feedback on how their technique affected the variability of their results. Figure 4 shows the improvement in pipetting competency after completing the training.

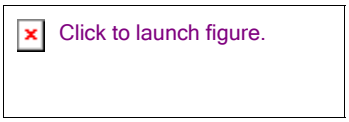

Figure 4: Improved Pipetting Competency Following Training

This study, performed in the scope of training, illustrates the need for pipette-technique training. Pipettes are easy to use properly, but there are specific procedures which, when followed, will improve pipetting accuracy and precision (Pentheny 1997). The training involved is minimal, and the results are potentially dramatic. There are few training programs a laboratory could undertake that will yield such meaningful benefits at such low cost.

Reducing Mechanical Pipette Error

The integrity of the data collected with any instrument in a laboratory may be called into question, and pipettes are no exception. Detecting a malfunctioning pipette may require examining data collected with that device since its last calibration. The sooner a failure is detected, the better.

The ideal way to detect pipette failures is to perform calibrations in the laboratory using the same operators working under the same conditions in which the pipettes function daily. This approach is optimal because it enables operators to evaluate the impact of pipetting technique and environmental factors, in addition to each pipette's state of repair, on the accuracy and precision of the calibration results. As a consequence, mechanical failures can be mitigated more effectively. In this way, operators and managers can be assured that pipette failure and improper pipetting technique are not significant sources of error in the laboratory.

Calibration Guidelines

The goal of every pipette-calibration program should be to help ensure accurate and precise liquid delivery under working-laboratory conditions. The suggestion that laboratories perform on-site calibration, using their own operators, follows from this goal. In addition, pipettes should each have a discreet identification number and be assigned to a specific individual or laboratory function. This will enable the identification of which

pipette was used for each assay performed. Scheduling calibrations at regular intervals helps ensure that it will happen in a busy laboratory. It is also useful to carry out interim performance verification checks before critical assays or any time damage to a pipette is suspected.

Another question that often comes up concerns the number of data points to take for calibration purposes. Ten data points are considered adequate to verify accuracy and precision (NCCLS 1984). A quick check using four data points may be used to verify accuracy alone but is statistically insufficient for evaluating precision (ASTM 1997). Variable-volume devices should be calibrated at the volumes at which they are commonly used in the laboratory. Testing across a range of volumes is essential to verify correct function for these devices. As shown by Pipette B in Figure 2, performance will degrade when using a variable-volume pipette at its lowest setting. It is, therefore, wise to specify limits on the use of variable-volume pipettes, so they are not used below 30 percent of their maximum volume.

When a failing pipette is detected, do not rush to adjust it. Pipettes fail for a reason, and it is desirable to identify the cause of the failure before attempting to fix it by adjusting the device. For example, if a pipette is underdelivering, it is likely the result of a leaking o-ring or seal. In this case, the pipette needs to be repaired. Adjustment at this point would only make matters worse.

Conclusion

Air-displacement pipettes are precision instruments that should not be taken for granted. Pipettes tend to fail silently and randomly, impacting sample and reagent delivery. Periodic calibration and preventive maintenance are, therefore, essential to ensure the integrity of laboratory results. Operator technique is also a significant source of pipetting error. However, pipetting-technique training, especially if it offers the opportunity to obtain immediate feedback, is easy to do and has a significant positive effect on performance.

Ten Tips for Improving Pipetting Technique

Operator technique has a major impact on pipetting performance. Following these ten tips (Artel, Incorporated 1998) will enable operators to obtain more accurate and precise results in laboratory conditions. The tips are listed in order of importance.

1. **Prewet the pipette tip**—Aspirate and expel an amount of the sample liquid at least three times before taking a sample for delivery. Evaporation in the tip can cause a significant loss of sample before delivery. Prewetting increases humidity in the tip, thus reducing the amount of and the variation in sample evaporation.
2. **Work at temperature equilibrium**—Allow liquids and equipment to equilibrate to ambient temperature. The volume delivered varies with air pressure, relative humidity, and vapor pressure of the sample; all of which are temperature dependent.
3. **Examine the pipette tip before dispensing**—Wipe the tip carefully and only if there is liquid on the outside. Otherwise, sample liquid may be wicked from the tip.
4. **Use standard-mode pipetting**—For all but viscous samples,

standard-mode (also called forward-mode) pipetting yields better accuracy and precision than reverse-mode pipetting. In reverse-mode, the plunger is depressed completely (e.g., past the first stop) to aspirate the sample.

5. **Pause after aspiration**—Pause with the tip in the liquid for one to two seconds after aspirating the sample. This is important because the liquid in the tip bounces slightly when the plunger stops.
6. **Lift the pipette straight out**—Do not touch the tip to the sides of the container. Surface tension causes the sample to vary if the exit angle varies, particularly for small volumes.
7. **Minimize handling of the pipette and tip**—Set the pipette down between deliveries. Body heat transferred to equipment during handling disrupts temperature equilibrium.
8. **Immerse the tip properly**—Immerse the tip 2-5mm below the meniscus and well clear of the container walls and bottom during sample aspiration, otherwise volume is affected.
9. **Use the correct pipette tip**—Securely attach a high-quality tip designed for use with the pipette and appropriate for the size of the container.
10. **Use consistent plunger pressure and speed**—Depress and release the plunger smoothly. Pipettes are precision instruments and give more consistent results when operated with care.

References

Artel, Incorporated. *Ten Tips to Improve Your Pipetting Technique*. Artel, Incorporated, Westbrook, Maine, 1998.

ASTM International. *Standard Specification for Piston- or Plunger-Operated Volumetric Apparatus*. ASTM E1154. ASTM International, West Conshohoken, Pennsylvania, 1997.

Conners, M. and Curtis, R. H. Pipetting error: A real problem with a simple solution. Part I, *American Laboratory News* (1999) 31(13):16-19.

Curtis, R. H. Controlling pipette performance in the real world, *Calibration Laboratory* (2000) 7(1):32-36.

Curtis, R. H. Performance verification of manual action pipettes. Part 1, *American Clinical Laboratory* (1994) 12(7):8-10.

Curtis, R. H. Performance verification of manual action pipettes. Part 2, *American Clinical Laboratory* (1994) 12(9):16-17.

ISO. *Piston-Operated Volumetric Apparatus: Piston Pipettes.* Part 2, ISO 8655-2. ISO, Geneva, Switzerland, 2002.

NCCLS. *Determining Performance of Volumetric Equipment: Proposed Guideline.* NCCLS, Wayne, Pennsylvania, 1984.

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