Reducing sources of error in pipetting

Introduction

One of the most widely used tools in laboratories today is the mechanical air-displacement pipettor. However, these mechanical action pipettors sometimes fail to deliver as accurately or precisely as they should. Often it is due to a mechanical failure in the pipettor itself (e.g. contamination, damaged sealing or out of calibration), but even more often the reason is simply human error. Depending on the failure, the user may not notice the error during pipetting leading to unexpected incorrect results. Still, in modern laboratory work the reliable measurement of samples, i.e. pipetting, is essential for the success of quantitative analysis. Particularly in highly sensitive tests a small mistake in pipetting can cause a large error in the final result. Therefore, automatisation of pipetting is increasing heavily with increasing demands for reliability and ease of work.

Inaccuracy and imprecision

To be accurate and precise in pipetting, one has to understand first the definitions. Precision is an agreement between replicate measurements. Precision has no numerical value, it is quantified by the imprecision. So high precision i.e. small imprecision, means very little variation between the repeated measurements on the same sample. On the other hand, it is possible to be very consistent, but consistently wrong. Inaccuracy is the numerical difference between the mean of a set of replicate measurements and the true value - so high accuracy i.e. small inaccuracy means a very little difference between your mean sample and the true value. What is needed, of course, is both precision and accuracy. To achieve both good accuracy and precision you require a precision instrument with quality tips, but you must also follow good laboratory practise - cleanliness and consistent correct handling.

Factors affecting pipetting performance

Reliability means good accuracy and precision. The first requirement is the precision instrument. In addition, there are many factors related not only to the pipettor, but also to consumable tips, working conditions, and the laboratory worker which affect pipetting. Recently, a remarkable study has been made to calculate the effect of the various factors to pipetting performance (Lohner et al., 1996). This study has been one of the key elements for creating new international standard for pipettors, namely the DIN 12650 (Deutsches Institut für Normung). The DIN 12650, which all pipettor manufacturers have to follow will become effective by the end of 1997 and first steps for this standard to become an ISO standard have already been taken. Different sources of error in DIN 12650-2 include mechanical faults in the pipettor, but also the fitting and properties of the tip, temperature (differences in temperature between the pipettor, fluid and the ambient temperature), air pressure, humidity, volume of air interface (dead volume), nature of the fluid, angle of pipetting, pre-wetting of the tip, and even the rhythm and speed of pipetting. Table I summarises the effect of various possible mistakes in pipetting. If thousands of pipettings are done daily, strain can cause additional errors. Moreover, choosing the right pipetting technique for a certain type of sample or application eliminates inaccurate results. Regular maintenance and checking of calibration guarantees that a pipettor performs according to specifications.

Pipetting techniques

Dispensing with mechanical air-displacement pipettors demands skills and experience to do it right. The most common pipetting techniques are forward pipetting, reverse pipetting, dispensing, sequential dispensing and diluting. While mechanical pipettors can be used for pipetting (forward and reverse), the Biohit Proline Electronic pipettor covers all these functions. It is critical to determine the pipetting technique best suited for each application because the choice of the technique can significantly affect the results of an analysis. By far the commonest method, forward pipetting, discharges all the liquid by one full movement of the piston. It is suitable for aqueous solutions containing small concentrations of protein or detergent. Pre-rinsing of the tip before the actual pipetting improves the results, but is very often skipped to save time and fatigue. For biological, viscous or foaming liquids, or very small volumes of liquid reverse pipetting improves the results significantly. In this technique, the protocol begins and ends with the tip containing liquid. Dispensing multiple aliquots of a single fluid is a universally used protocol. An electronic pipettor is far more efficient, safe and accurate for this purpose because it allows repeat dispensing from each filling, reducing the number of sample to vessel actions and so reducing sample contamination risk and pipettor tip usage. Such repetitive action using a mechanical pipettor can contribute to Carpal Tunnel Syndrome and other industrial ailments. Dilution and sequential dispensing techniques are generally only possible with an electronic pipettor. In sequential dispensing a series of different volumes can be delivered in any desired order, a most useful technique in serology work and related applications. In dilution technique, the first volume is aspirated, followed by an air gap then the second volume is aspirated: the two are then dispensed in one action (Fig 1). This technique improves throughput and reduced fatigue when it replaces the double operation of a mechanical pipettor - especially in a large volume or multichannel pipettors.

Figure 1. Automatic dilution of samples in pipettor tip using Biohit Proline Electronic pipettor saves time and replaces the double operation of a mechanical pipettor.

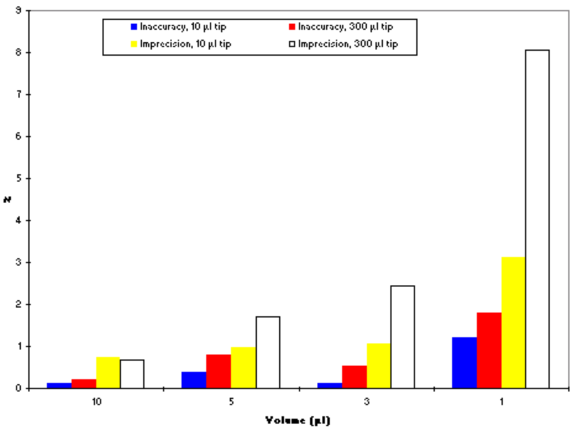

Figure 2. Comparison of inaccuracy and imprecision using 10 and 300 µl tip in dispensing 1, 3, 5 and 10 µl.

There are a few things not depending on the technique one should always pay attention to when pipetting:

* The pipettor/tip should be chosen to minimise the air space between the piston and the liquid (Fig.2).
* The tip should not be placed too deep, but just under the surface of the liquid in the reservoir (2-3 mm).
* The tip should touch the vessel when withdrawed to avoid extra liquid outside the tip
* Pre-wetting the tip for 2-3 times improves both accuracy and precision.
* The pipettor should be held vertically, not at angle.
* The aspiration should be done smoothly, not too quickly.

Figure 2. shows how air space between the piston of the pipettor and the liquid affects the results. 1, 3, 5 and 5 µl were pipetted using Biohit Proline Mechanical pipettor 0.5-10 µl with either 10 µl or 300 µl tip (2-in-1 pipettor). Distilled water was pipetted ten times using reverse pipetting and the imprecision and inaccuracy were calculated. Volumes of smaller than 10 µl were more

accurate with 10 µl than 300 µl tip. The lower the volume the greater the mistake. Especially the imprecision was a lot worse with all the volumes when using a large tip (300 µl) compared to a smaller tip (10 µl). In all volumes both accuracy and precision were better using an electronic pipettor due to the fact that an electronic pipettor performs identically independent on user (data not shown).

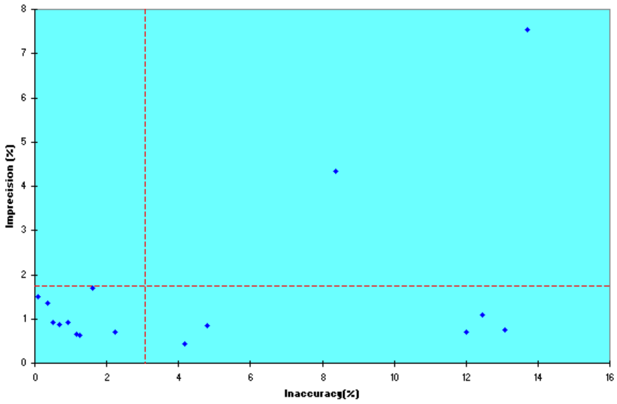

Figure 3. Inaccuracy and imprecision of 15 different manufacturer's tips using Biohit Proline Electronic pipettor. Inaccuracy and imprecision specifications (DIN 12650) are marked as dashed lines.

The pipettor tip

Pipetting with disposable pipettor tips has become an integral part of many different applications in liquid handling. First, disposable tips provide a level of protection from infectious materials and radioactive substances saving the use of aggressive cleaning agents. Second, many tests for example in the field of molecular biology have only become possible with the invention of piston-stroke pipettors and plastic disposables. The tip's shape, material properties and fit have a considerable influence on the accuracy of liquid handling. What one should look for in pipettor tips is that they are manufactured without any contaminating chemicals, they are clean and free from dust particles, uniform in size and geometry for leak-free attachment to the tip cone. In addition, the surface of the tip should be smooth and regular to prevent retention of the liquid and the opening flash free to prevent the formation of droplets. The tip material should also have good chemical resistance. To ensure accurate pipetting results, only tips specified by the manufacturer should be used. Cheap, poorly fitting tips not designed for a pipettor can result in serious measurement errors. Especially the performance of filter tips used in wide variety of applications vary a lot depending on the pore size and material of the filter in addition to the properties of the tip. Figure 3 describes the performance (inaccuracy and imprecision calculated from 10 subsequent pipettings with each tip using gravimetric analysis) of fifteen different tips from different manufacturers. As shown, in this test only nine tips out of fifteen perform according to the specifications (DIN 12650) (dashed line). Therefore, if using others than tips specified by the manufacturer, one should always test the performance before beginning the analysis. Saving money in tips may result additional costs in need for reanalysis because of pipetting errors. One should also keep in mind that there is no such product as a universal tip.

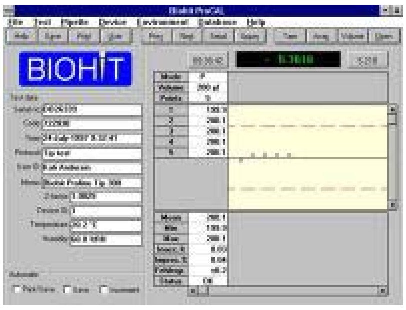

Figure 4. Performance checking of a pipettor using the gravimetric method monitored by a computer.

Environmental conditions

Sources of error from the environment include temperature (differences in temperature between the pipettor, fluid and the ambient temperature), air pressure, and humidity. The single greatest contributor to error is temperature, especially if working with air displacement pipettors (Joyce and Tyler, 1973; Lohner et al., 1996). As an example, increasing the temperature of the liquid from 5°C to 28°C while other elements (pipettor and tip) are kept constant (22°C), pipetting of 1 ml can have up to 6% error in volume. An ideal environment for pipetting maintains ambient temperature within 1°C, including all parts of the liquid handling system.

Mechanical faults in pipettor and calibration

Malfunction of the pipettor can cause errors in numerous ways. Therefore, performance checking and service should be done regularly for all pipettors. Performance checking can be done using a gravimetric or colorimetric method. The gravimetric method involves weighing of samples of distilled water at room temperature using a reliable electronic microgram balance with a readability of 0.1 mg. Most often a computer software is used to record the results and calculate the inaccuracy and imprecision (Figure 4).

Recalibration is required at some point for all mechanical pipettors. For some pipettors it can be done easily and quickly, for some it is a complicated procedure. However, there is now a range of products on the market that require no calibration, the Biohit Proline Electronic Pipettor range. Calibration is not needed because in the patented construction of Biohit Proline electronic pipettors (Suovaniemi O. and Ekholm P., 1994), a fast DC motor moves the piston and its movements are monitored with optical feedback in real time under microprocessor control (Fig.1). In other words, the pipettor controls its own performance.

Summary

With experienced personnel following good laboratory practise and in an ideal environment (constant temperature for air, pipettor, tips and the liquid, constant humidity and air pressure) most of the pipetting errors can be avoided. Sometimes the environment is impossible to control, but as long as the operator is aware of the possible factors affecting the pipetting performance, unexpected results can be avoided. To ensure adequate accuracy, precision and correct performance, pipettors should be checked regularly and calibrated when needed. The great advantages of the electronic pipettors are not only their self-calibration, but also the ease of use, the wide field of applications, and high reproducibility of pipettings due to their well defined piston movement and automatic control which reduces human error. Special attention has also been paid to ergonomic design. The result is less strain, while preserving the control feel of the mechanical pipettor. The high quality of the results obtained with electronic pipettors is, however, very difficult to achieve with manually operated pipettors.

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