11. Pipetting performance. The critical factor for reliable analysis.

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In both research and routine laboratory work the reliable measurement and dispensing of samples and reagents, i.e. pipetting, are essential for the success of quantitative analysis. Traditionally, pipetting has been done almost exclusively by suction using glass pipettes. Single- and multichannel mechanical pipettors and disposable tips were developed by the end of 1960s. Today, mechanical air-displacement pipettors belong to the standard equipment of all laboratories. However, new demands on more and more convenient and accurate pipetting devices have resulted the development of electronic pipettors and fully automatic liquid handling systems.

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 environment, and the laboratory worker, which affect pipetting. Even experienced users may obtain incorrect measurements from time to time and if thousands of pipettings are done daily, strain can cause additional errors. Electronic pipettors (Figure 1) are considered to be more accurate because of their well-defined piston movement and automatic control, which reduces human error. It is important to evaluate and to reduce, wherever possible, both random and systematic errors in liquid sample handling. Regular maintenance guarantees that the pipettor performs according to specifications.

Figure 1. Dispensing of aliquots using automated instruments, like Biohit Proline Electronic pipettor reduces human error.

Inaccuracy and imprecision

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. To achieve it you require a precision instrument, but you must also follow good laboratory practice - cleanliness and consistent correct handling.

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. Accuracy is achieved by careful calibration of a precision instrument. What is needed, of course, is both precision and accuracy.

Factors affecting pipetting performance

The pipettor tip

The tip is an integral component of the pipetting system and its shape, material properties and fit have a considerable influence on the accuracy of liquid handling. In addition to fitting, most important is to test how the tip wets, and whether there are droplets remaining after the sample is dispensed. To ensure accurate pipetting results, only tips specified by the manufacturer should be used. Cheap, poorly fitting tips not designed for the pipettor can result in serious measurement errors. If using others than tips specified by the manufacturer, one should always test the performance before beginning the analysis. 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. One should also keep in mind that there is no such product as a universal tip.

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.

Pipetting techniques

Dispensing with mechanical air-displacement pipettors demands skills and experience to do it right. 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 minimize the air space between the piston and the liquid.
* The tip should not be placed too deep, but just under the surface of the liquid in the reservoir (2-3 mm).
* Pre-wetting the tip improves both accuracy and precision.
* The pipettor should be held vertically, not at angle.
* The aspiration should be done smoothly, not too quickly.

The most common pipetting techniques are forward pipetting, reverse pipetting, dispensing, sequential dispensing and diluting. Choosing the right pipetting technique improves accuracy. While mechanical pipettors can be used for pipetting (forward and reverse), the Biohit Proline Electronic pipettor covers all these functions. By far the most common 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. 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 (Figure 1). 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.

Calibration and performance checking

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 2).

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. In other words, the pipettor controls its own performance.

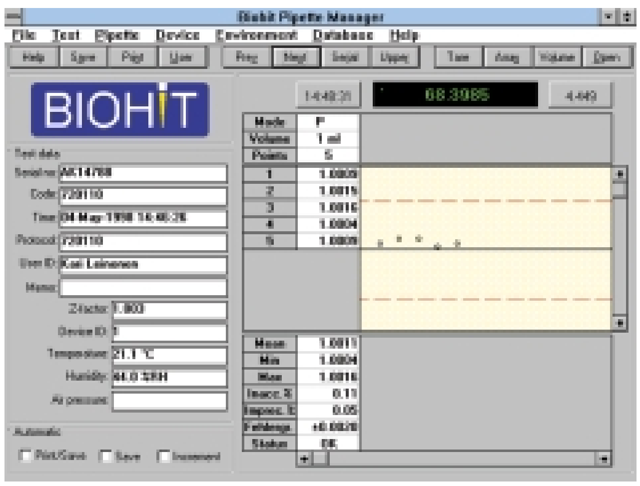

Figure 2. Performance checking of a pipettor using the gravimetric method monitored by a computer.

Summary

With experienced personnel following good laboratory practice 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. 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, high reproducibility of pipettings and the wide field of applications as a result of their integrated dispensing functions. The high quality of the results obtained with electronic pipettors is very difficult to achieve with manually operated pipettors.

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