Optimizing your pipetting performance

It is no longer possible for any field of the natural sciences and medicine to function without being able to dispense minute amounts of liquid. Pipetting in a microlitre range has become a permanent and indispensable component of a large number of experimental methods. Current analytical methods in clinical chemistry and industry demand highly precise liquid handling processes in the microlitre and millilitre range. At the same time, new techniques, such as genetic engineering place increasing demands on the design, construction and materials of these systems.

One of the most common activities of laboratory workers is primary sample or reagent addition whilst performing a test. To accurately measure a specified amount of fluid a pipettor is generally used. The risks associated with handling fluids are well known, but there are still physical risks to the laboratory worker. Some laboratory workers perform thousands of tests daily with problems being caused by continual depression of the plunger on pipettors, which in turn cause errors in pipetting. In addition, there are many factors related to the pipettor, tips and the laboratory worker which affect the accuracy and precision in pipetting.

How then can we assure accurate and precise pipetting performance?

First of all, we have to understand what accuracy and precision in fact mean. 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 practise - 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. To achieve this you need: a quality pipettor, quality tips, regular maintenance and consistent handling.

A large number of different devices for sample transport and preparation are now available to scientists and lab personnel. In order to make rough classification, a distinction is made between pipettors, dispensers and multi-functional dispensers. These instruments function either with air or positive displacement principle. In addition, a distinction is made between fixed volume pipettors (e.g. 100 µl) and variable volume pipettors (e.g. 0.5 µl to 10 µl), which are available either as single or multichannel versions. Nowadays, pipettors can also be divided to mechanical and electronic semi-automated pipettors.

The tip is an integral component of the pipetting system and it shape, material properties and fit have a considerable influence on the accuracy of liquid handling. 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. One should keep in mind that there is no such product as a universal tip.

Regular care and maintenance guarantees long lifetime of the pipettor. How often then should a pipettor be checked?

This clearly depends on your own laboratory practice: on acceptable tolerances, on volume and pressure to use, and the critical nature of the work. Every pipettor should be regularly checked by a competent operator. A thorough test - say monthly or quarterly - should involve at least ten samples being stringently checked by the gravimetric method.

Recalibration, which usually involves weighing of samples of distilled water at room temperature using a reliable electronic microgram balance with a readability of 0.1 mg, 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

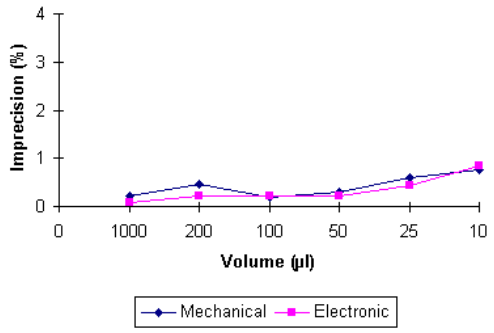
because the electronic pipettors use microprocessor control with optical feedback to monitor piston movement.

The most difficult demand for accurate and precise pipetting results is the consistent handling. There are number of factors that influence the pipetting performance: The quality of the products and tips, previously discussed, human error, pipetting techniques, strain (Repetitive Strain Injuries) etc., and other factors, such as environment (temperature, air pressure, humidity), type of sample and type of application. The following applications describe some of these problems and presents solutions for them.

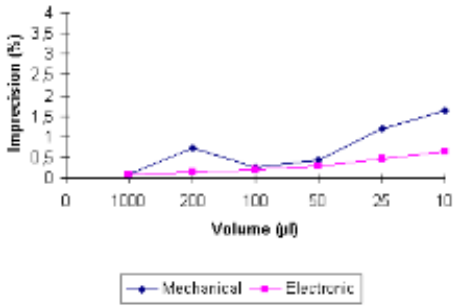
The effect of user experience on pipetting precision

Nine persons were divided in three groups according to their experience in pipetting. Each person pipetted distilled water with the same Biohit Proline mechanical and electronic pipettors (P-mode). The imprecision of ten subsequent results of volumes 10, 25, 50, 100, 200 and 1000 µl was recorded and the results are detailed below.

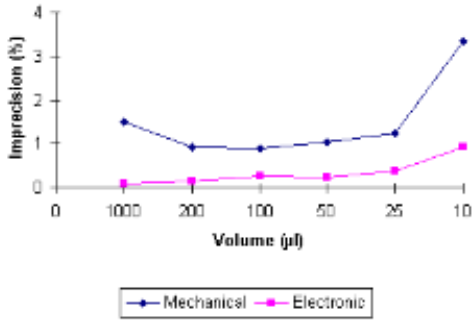
Experts



Moderate experience



No experience

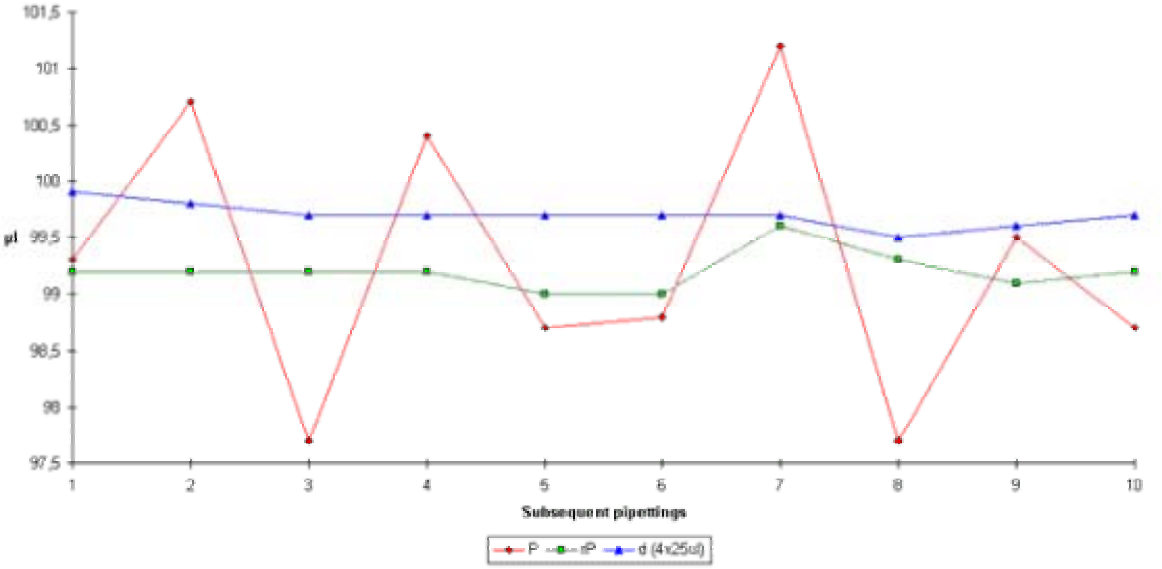


Conclusion:

The electronic pipettors produce precise results independent of user experience, but experience and good pipetting practise are essential for reproducible results with mechanical pipettors.

How does pipetting technique affect the accuracy and precision when viscous or protein rich solutions?

75% glycerol was used as a test sample and the volume of 100 µl was pipetted/dispensed using different modes of the Biohit Proline Electronic Pipettor. Ten subsequent results were recorded and the results are detailed below.



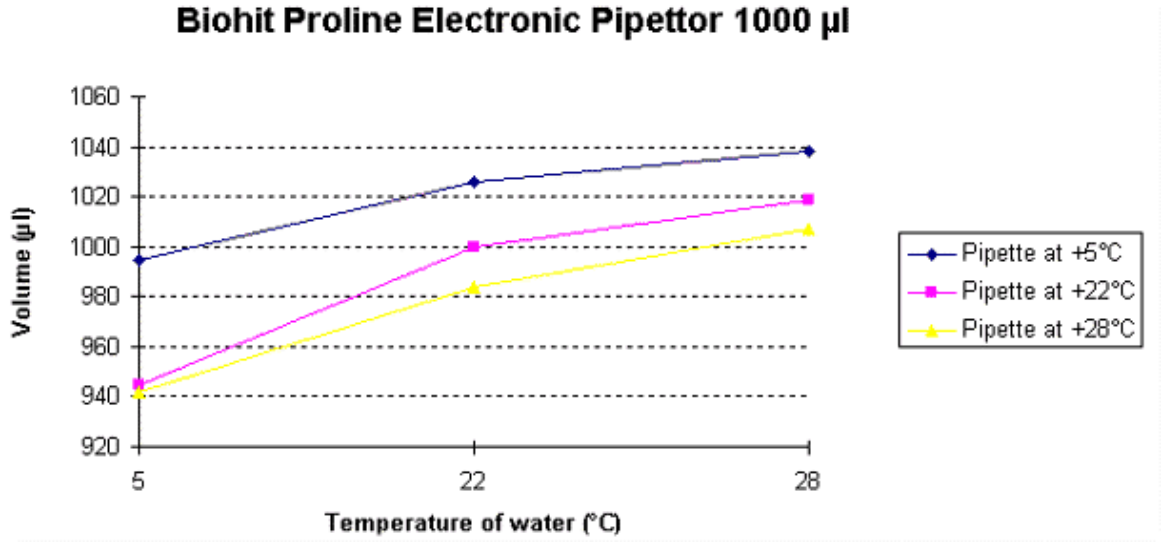
Conclusion:

The best accuracy is obtained by dispensing (d) 100 µl (4 x 25 µl) allowing the viscous solution to slowly run out of tip. However, also the reverse pipetting mode (rP) yields satisfactory results, whereas the normal pipetting mode (P) is not recommended for this application.

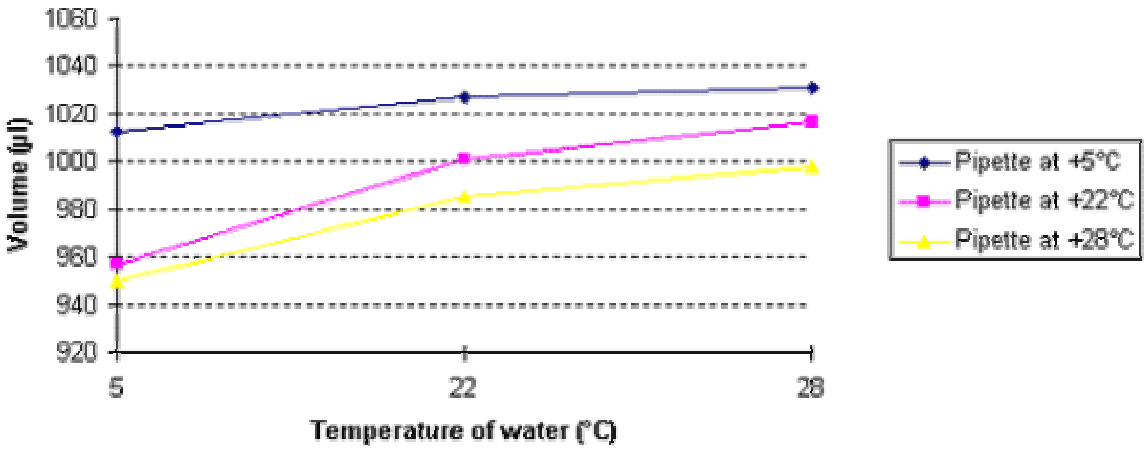
Similar results were obtained with serum samples (data not shown).

The effect of temperature on pipetting accuracy

All air displacement pipettors are sensitive to temperature differences between sample and environment (pipette and tips). Biohit Proline Electronic Pipettor 1000 µl and mechanical pipettor 200 -1000 µl were stored sequentially at temperatures of +5°C, +22°C and +28°C for several hours and tested after each temperature with distilled water stored at different temperatures. Pipettors were tested pipetting ten times 1000 µl to the balance and the mean values are shown below.



Biohit Proline Mechanical Pipette 200-1000 µl



Conclusion:

The smaller the difference in temperature between the pipettor, tip and pipetted solution is, the more accurate the results are. For accurate results, keep the pipettor, tips and the liquid at the same temperature.

New demands on pipettors and related dispensing devices have resulted in a multitude of different models. More and more convenient systems for automated or semi-automated pipetting and dispensing are being developed because of the need to handle increasingly larger sample series with smaller volumes. More recently, the pipetting ergonomy has become an important feature with increasing incidence of repetitive strain injuries among workers using excessive and repetitious movements of the upper extremities.

Electronic pipettors which enable the user to process samples with a level of reproducibility which can not be matched by mechanical pipettors, often form a basis for successive and additional automation. Moreover, the ergonomy of an electronic pipettor is exceptional compared to mechanical pipettors. When the pressure needed to aspirate fluid with mechanical pipettors ranges from 800 gms to over 2.5 kg, dependent on pipette type and volume (with blow-out functions over 5 kg!), the electronic pipettor needs a pressure of approximately 150 gms for both aspirate and blow-out. These are not the only benefits provided by Biohit Proline Electronic Pipettors; they can also mix samples, dilute and dispense and have the ability to control the aspirate and dispense speed. Multi-functional dispensing systems, like Biohit Proline Electronic Pipettors, are a further step towards automation and ergonomy with improved accuracy and precision.

For further information circle the reader card below to receive the Biohit Guide to Liquid Handling.

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