Choosing a pipetting technique affects the results of your analysis

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Accurate and precise pipetting performance is critical in laboratory analysis, particularly in highly sensitive tests where a small mistake in pipetting can cause a large error in the final result. Therefore it is of great importance to evaluate and to reduce, wherever possible, both random and systematic errors in liquid sample handling. 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. Regular maintenance guarantees that your pipettor performs according to specifications.

The first step in pipetting is to choose the right pipetting technique best suited to the type of work. 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. 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 (Figure 1). 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. 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.
Sequential dispensing mode of the Biohit Proline Electronic pipettor. In this mode a series of different volumes can be delivered in any desired order.

All air displacement pipettors are adjusted to give their nominal volume in a reference temperature and with distilled water as a test liquid. The viscosity and density of the pipetted liquid affect the results.

Pipetting Human Whole Blood Samples

Biohit Proline Electronic (BPE) pipettors (0.2-10 µl with microtip 10 µl, 5-100 µl and 10-250 µl with 300 µl tip and 20-500 µl with 1000 µl tip) were used to pipette human whole blood samples (pool of four blood samples with a density of 1.0392 g/ml) using forward pipetting (P), reverse pipetting (rP) and dispensing (d) techniques (modes). The dispensing mode was programmed to aspirate twice the desired volume and the second volume was taken for analysis.

To simulate the practical working situation a new dry tip without any pre-rinsing was used for each aspiration-delivery cycle. After aspiration the outside of the tip was wiped against the test tube edge. The delivery was done against the wall of the receiving vessel. The weight of the volume conversion were done automatically by the Biohit Balance Reading program. The temperature of the pipettors, tips, and the blood sample were identical and constant during the test period (24°C). Every test series consisted of ten subsequent pipettings. The results are shown in Figures 2 and 3.

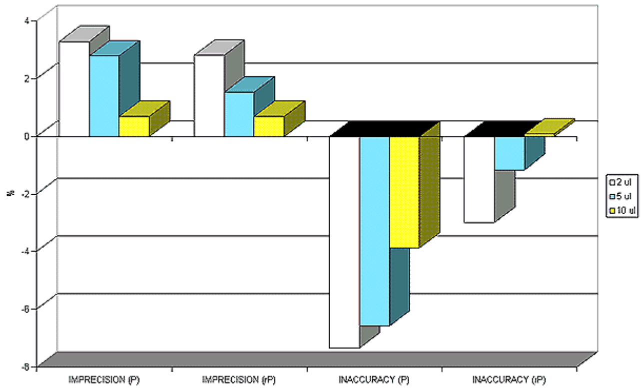

Figure 2.
The inaccuracy and imprecision values of 10 subsequent pipettings of 2, 5 and 10 µl using forward pipetting and reverse pipetting mode of an electronic pipettor.

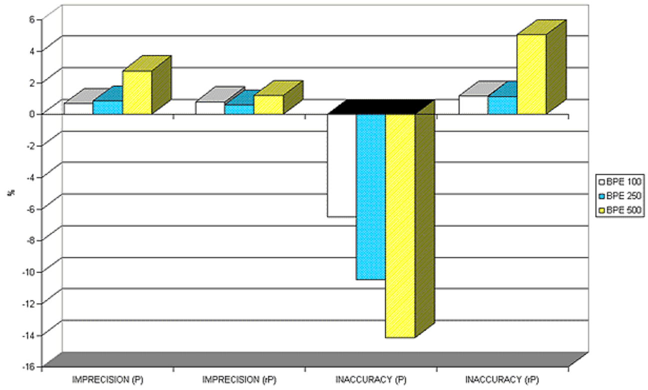

Figure 3.
The comparison of inaccuracy and imprecision values of different electronic pipettors (300 µl vs. 1000 µl tip) delivering 50 µl using forward pipetting and reverse pipetting technique.

Imprecision values were quite good in all volumes, pipetting modes and with different pipettors. The accuracy was constantly better when the reverse pipetting mode was used, compared to the forward pipetting mode. Even better accuracy values were obtained, when a special application of dispensing mode was used. Pipettor BPE 10 gave surprisingly good results even when as small as 2 µl volume was pipetted. The air volume inside the tip and pipette is very small in this pipette and the tip orifice seemed to be suitable to dispense small volumes of whole blood. Pipettors BPE 100 and BPE 250 use the same 300 µl tip and there was no notable difference in the results either. Pipettor BPE is used with 1000 µl tip and the volume tested in this work (50 µl) were small compared to the whole working range of that pipettor, so the air volume was quite large. Also the results were poorer than the same volumes tested with the smaller pipettors. Similar results were obtained with serum samples (data not shown). Always when reverse pipetting technique is used and also otherwise when small volumes are pipetted the delivery has to be done against the surface of the receiving vessel. This touching has to be done as identically as possible for every pipetting cycle.

This is easy to carry out when electronic pipettors are used, because no manual force is needed for the delivery operation.

Conclusion

This study was done to see, what are the performance characteristics for relative small volumes of whole blood samples, when electronic pipettors with different working ranges are used. The reverse pipetting technique is the method of choice when whole blood samples are pipetted. For research purposes or when the maximal accuracy is needed the special application of the dispensing quality of the BPE-pipettors may be useful. On the other hand, the smaller the tip i.e. the smaller the air volume, the more accurate the results are. It has to be pointed out that even better results are obtained, when the tips are rinsed in the sample liquid before sampling.

Pipetting solvents

Solvents with high vapour pressure (e.g. chloroform) can not be dispensed with an air-displacement pipettor with the accuracy and precision specified for distilled water. An error results due to the evaporation of the solvent during aspiration. A thin film of the liquid usually remains in the tip or the pressure of the vapour pushes the liquid out of the tip before the actual delivery. As a result, generally, the volume dispensed is too small. The accuracy of dispensing can, however, be improved by pre-wetting the tip several times with the solvent before the actual pipetting. Minimising the air space between the solvent and the piston of the pipettor, i.e. using as small a tip as possible improves the results also significantly.

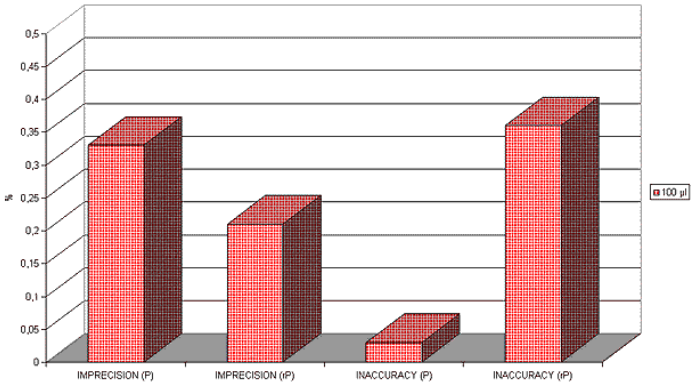

Figure 4.
The results of pipetting methanol using forward and reverse pipetting technique.

Biohit Proline Electronic (BPE) pipettor 5-100 µl was used to pipettemethanol (a density of 1.2658 g/ml) using forward pipetting (P), reverse pipetting (rP) technique. The tip was pre-rinsed 3 times before the actual pipetting. The results are shown in figure 4.

Conclusion

Methanol can be pipetted with reasonable accuracy and precision using air-displacement pipettor. In this application, reverse pipetting did not improve the results, but on the contrary gave poorer inaccuracy than forward pipetting. Especially when large volumes (ml range) are pipetted, forward pipetting with pre-rinsing is the best choice. It has to be pointed out, that pipetting solvents is always a difficult task and each solvent has to be tested individually whether it can be pipetted with air-displacement pipettors or not. One should also make sure that the pipettor itself is resistance to the chemicals used. The piston and piston seal may require lubrication with silicone grease if solvents are pipetted frequently. Some of the solvents can, however, results damage to the sealing or other parts of the pipettor. Chemical resistance sheets are available at least for Biohit Proline pipettors.

Prospects

Microprocessor controlled instruments are finding favour in many laboratories worldwide in response to increasing workloads and the spread of occupational health hazards for upper limbs such as Carpal Tunnel Syndrome. The great advantages of the electronic pipettors are the high reproducibility of pipettings and the wide field of applications as a result of their integrated dispensing functions that would be impossible to achieve with manually operated pipettors. Moreover, once set, the speed and the movement are independent of the operator, reducing the variability and allowing concentration to be focussed where it is most needed. The ease of use and high performance of Biohit electronic pipettors make them superior in today?ßs laboratory applications.