3. Factors influencing pipetting performance: The benefits of an electronic pipettor

Only a couple of decades ago pipetting was carried out almost exclusively by suction using glass pipettes. Today, mechanical air-displacement pipettors belong to the standard equipment of modern laboratories. Single-and multichannel pipettors were developed by the end of 1960s, and now, since the beginning of 1990s, new air-displacement pipettors are purchased, or used ones replaced, at a rate of more than one million units per year. Since 1960s, there has been a continuous stream of development and technical improvements in the field of liquid handling. New demands on more and more convenient and accurate pipetting devices have resulted the development of electronic pipettors and fully automatic liquid handling systems. Yet, mouth suction glass and plastic pipettes are still used in many laboratories.

Air-displacement pipettors are designed for convenient dispensing of fixed or adjustable volumes and cover large volume range. In these devices, a specified volume of air separates the sample - aspirated into a plastic tip - from the piston inside the pipettor. The delivered volume is determined by the piston displacement and the so-called "additional volume". There are many factors related not only to the pipettor, but also to tips, environment, and the laboratory worker which affect pipetting. Even experienced users may obtain incorrect measurements from time to time. Therefore it is important to evaluate and to reduce, wherever possible, both random and systematic errors in liquid sample handling.

Sources of error which affect the pipetting performance are either wrong adjustment, calibration, environment, or variations exerted by the operator. These include temperature (differences in temperature between the pipettor, fluid and the ambient temperature), air pressure, humidity, volume of air interface (dead volume), density of the fluid, angle of pipetting, pre-wetting of the tip, and even the rhythm and speed of pipetting. In addition, 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.

Pipettor tips

The tip is the final link delivering each precise measure to its destination. It`s shape, material properties and fit have a considerable influence on the accuracy of liquid handling. Quality tips are constructed from autoclavable, non-wettable premium grade virgin polypropylene without any contaminating chemicals. The surface of the tip is smooth and regular to prevent retention of the liquid and the opening flash free to prevent the formation of droplets. They are clean and free from dust particles and have good chemical resistance. To ensure accurate pipetting results, only tips specified by the manufacturer should be used. One should keep in mind that there is no such product as a universal tip. Saving money in tips may result additional costs in need for reanalysis because of pipetting errors. If using others than tips specified by the manufacturer, one should always test the performance before beginning the analysis. However, many manufacturers provide cross reference sheets indicating which tips fit to their pipettors.

Pipetting

Dispensing with mechanical air-displacement pipettors is thought to be easy. However, it demands skills and experience to do it right. The pipettor/tip should be chosen to minimise the air space between the piston and the liquid. For example volumes less than 10 µl are more accurate using a 10 µl tip than a 300 µl tip (Figure 1.), the difference increasing in both inaccuracy and imprecision with decreasing volume.

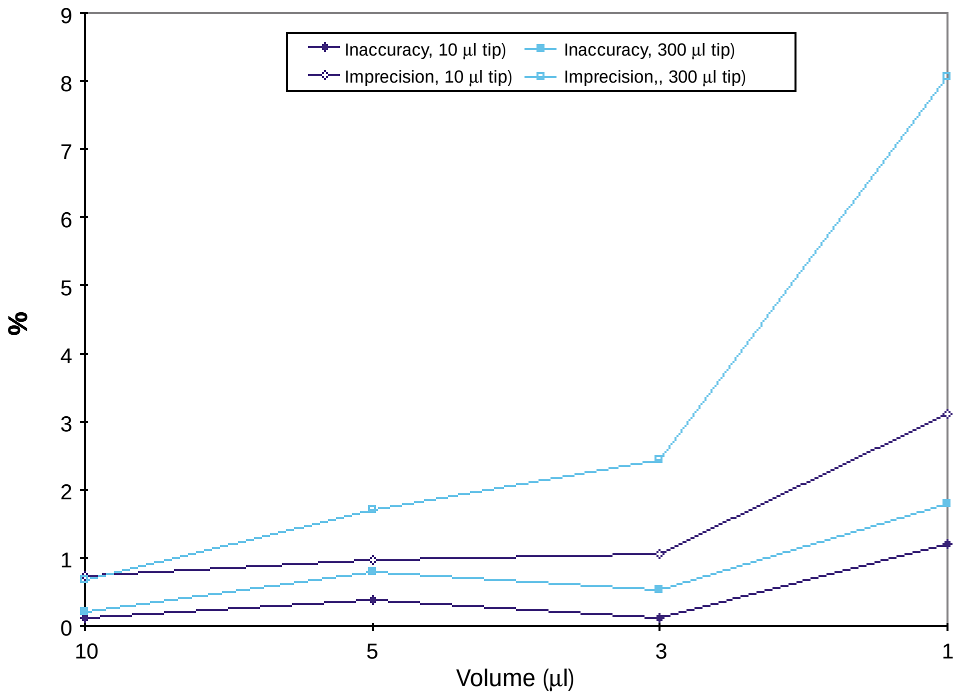


Figure 1. Inaccuracy and imprecision values of ten subsequent pipettings of 1,3,5 and 10 µl of distilled water (22°C) using Biohit Proline 10 and 300 µl tips with the same Biohit Proline 0.5-10 µl mechanical pipettor (2 in 1 pipettor).

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. On the other hand, if the tip is pre-wetted for too long (e.g. 5 minutes) before the actual pipetting, the first pipettings are too big (Lohner et al., 1996). The best results are obtained if the tip is touched against the the edge of the reservoir to remove the excess of the liquid when withdrawing the tip from the liquid. In addition, the pipettor should be vertical during the pipetting event. If held at angle, the height of the liquid column in the tip decreases and leads to aspiration of more liquid. Lohner et al. (1996) found 0.3% error in pipetting with 45° angle and 0.5% error with 60° angle using 1 ml pipettor. With larger volumes, the error is even bigger. If the tip is dipped too deep (3-4 cm) at the same time, the error can be more than double.

In the pipetting process, particularly aspiration requires both care and patience, because it must not occur too quickly. If it does, especially with volumes exceeding 100 µl, aspiration of air bubbles replacing some of the sample volume often results, yielding significant errors. In addition, according to Lohner et al. (1996) the rhythm of pipetting using mechanical pipettors is a potential source of error. It varies, not just from person to person, but from pipetting to pipetting. Depending on the pipettor, up to 0.7% error can occur. If thousands of pipettings are done daily, strain can cause additional errors. Electronic pipettors are considered to be more accurate because of their well defined piston movement and automatic control which reduces human error.

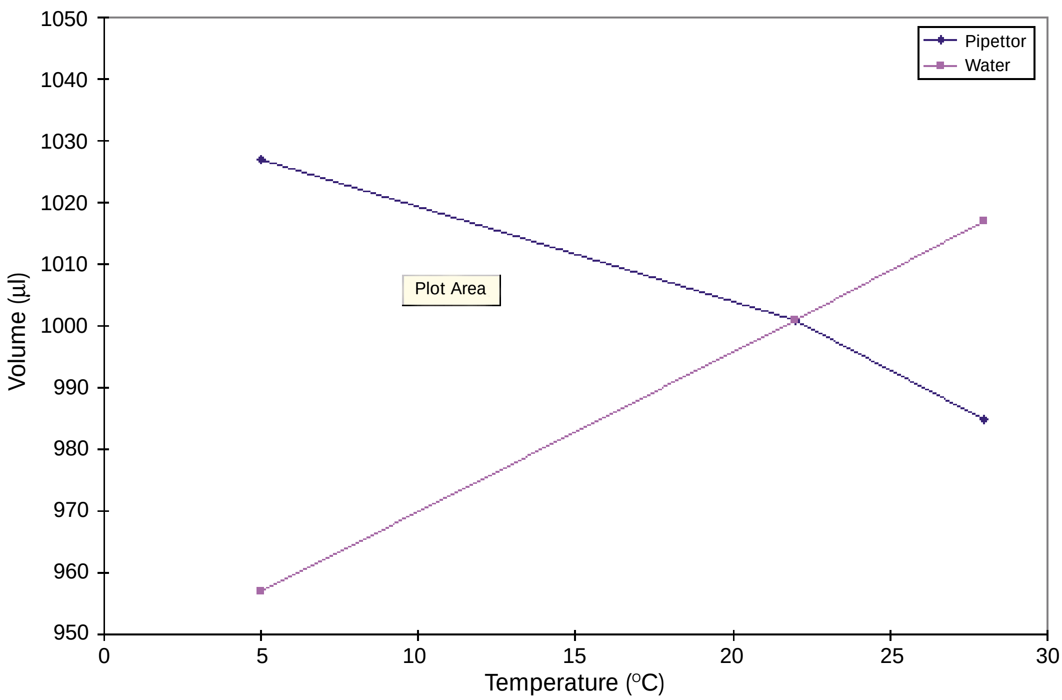
In the patented construction of Biohit Proline electronic pipettors (Suovaniemi O. and Ekholm P., 1994), a fast DC motor moves the piston. The piston movements are monitored with optical feedback in real time under microprosessor control. In other words, the pipettor controls its own performance. Moreover, the speed can be set to meet the requirements of the sample (Suovaniemi O., 1994). Therefore, errors due to the user differences in rhythm, speed, strain, and experience are eliminated (Figure 2.).

Figure 2. The Biohit Proline Electronic pipettor (A) and its operational principle (B).

Environment

Already in 1970s (Joyce et al., 1973) it was noticed that all air-displacement pipettors are sensitive to temperature. Not only the temperature of the environment, but also of the pipettor, tips and the liquid affects the performance. When the temperature of the pipettor increases from 5°C to 28°C (tips, liquid and environment 22°C), the delivery of 1000 µl varies up to 4%. If other elements are kept constant (22°C), but the temperature of the liquid increases from 5°C to 28°C, the difference is even bigger (6%). Notable is that increasing the temperature of the pipettor, the volume decreases, but increasing the temperature of the liquid, the volume increases (Figure 3.). The increase of the temperature of the air affects the pipetting performance least (1.5%). In most of the pipettors warm hands do not have an effect. If however the isolation of the piston from the handle (space between) is too small, warming the pipettor can cause large errors. In addition to temperature, humidity has an effect on pipetting. According to Lohner et al (1996) if the humidity in the room changes from 80% to 20%, the volume decreases 0.3-1.2% with 200 µl depending on the pipettor used. With bigger volumes the error is even bigger.

Figure 3. The effect of temperature on pipetting performance. 1000 µl of distilled water was pipetted ten times using Biohit Proline mechanical 200-1000 µl pipettor. The liquid and pipettor were stored at 5°C, 22°C and 28°C several hours before testing. The mean values are shown.



Conclusion

As a summary, in an ideal environment (constant temperature for air, pipettor, tips and the liquid, constant humidity and air pressure) and with experienced personnel most of the pipetting errors can be avoided. In practise liquids and environment vary a lot. For example in molecular biology work the enzymes must be pipetted ice-cold to avoid inactivation, but the pipetting is done at room temperature. On the other hand, the inaccuracy in pipetting is usually corrected by overdosing the enzyme. As long as the user is aware of the possible sources of error, most of the mistakes can be eliminated. However, in response to increasing workloads and demand for higher accuracy microprocessor controlled instruments are finding favour in many laboratories worldwide. The great advantages of the electronic pipettors are the ease of use, 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.

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