Liquid Handling Today

Fundamentals of Dispensing

Dispensing systems function according to two different physical principles: dispensing of liquid either takes place via an air cushion or by positive displacement. These two different dispensing principles are presented below taking piston stroke pipettes as an example. Important aspects of ergonomics are discussed as well.

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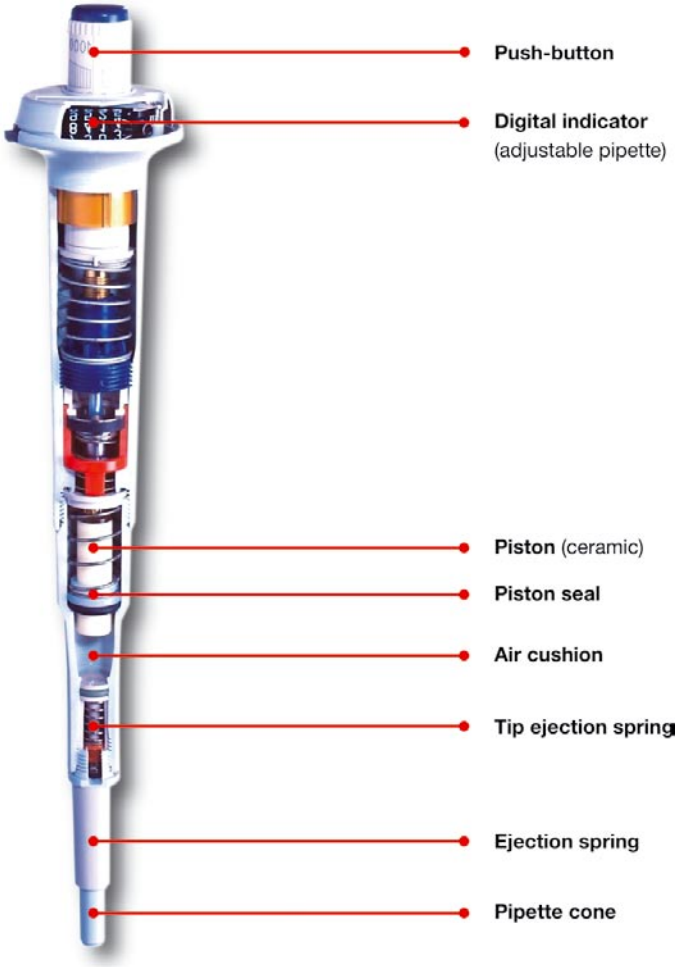

Fig. 1: Cutaway model of an air-cushion pipette (Eppendorf Reference)

Air-Cushion Principle (Air Displacement)

Air-cushion pipettes consist of a piston-cylinder system which performs the actual measurement (Fig. 1). An air-cushion separates the sample aspirated into a plastic tip from the piston inside the pipette. Upward movement of the piston produces partial vacuum in the tip, causing the liquid to be drawn into the tip. The air-cushion moved by the piston acts like an elastic spring from which the volume of liquid in the tip is suspended. Due to expansion of this air volume, the volume moved by the piston is approx. 2% to 4% greater than the aspirated volume of liquid required. Such expansion is compensated for by a factor that takes into account the dead volume and the lift height in the tip of the pipette. The influences of temperature, air pressure and humidity must be minimised with an air-cushion pipette through design measures so that the dispensing accuracy is not impaired.

Figure 2 shows the design principle of the air-cushion pipette based on the rest position (1). To prepare for aspiration of the liquid (2) the push-button is pressed to the first stop (measuring stroke). The piston moves down, displacing a volume of air that corresponds to the selected aspirating volume of the liquid. To aspirate liquid (3) the pipette tip is immersed into the liquid vertically. As the push-button slowly moves back, a partial vacuum is created in the pipette tip, aspirating the required volume through the tip opening.

To dispense liquid (4), the push-button is slowly pressed to the first stop (measuring stroke). The piston moves down, emptying the tip. To empty the tip completely ("blow-out", 5), the push-button is pressed to the second stop (blow-out), and the pipette is raised with the push-button still pressed wiping it against the wall of the vessel. After the push-button has moved back, the piston returns to the rest position (6).

The immersion depth of the pipette tip has a significant effect on the result. If the tip is immersed too deeply into the liquid, drops form on the outside, possibly falsifying the dispensing volume. If the tip is not immersed deeply enough into the liquid, turbulence is produced, and an incorrect volume will be aspirated.

Principle of Positive Displacement

Dispensing systems operating according to the principle of positive displacement are subject to physical influences other than those occurring with the air-cushion systems described above. The effects of an air-cushion are not applicable here so that these devices are also suitable for liquids and applications which can be seen as critical in conjunction with air-cushion systems. Such applications include: liquids with high vapour pressure, high viscosity or high density and applications in molecular biology such as the polymerase chain reaction, which calls for an absence of aerosols to prevent cross-contamination.

The dispensing accuracy of positive-displacement dispensing systems depends on the disposable plastic tip to an even greater extent than with air-cushion systems. Unlike the plastic tips of the air-cushion systems, the tips of the positive-displacement systems have an integrat-

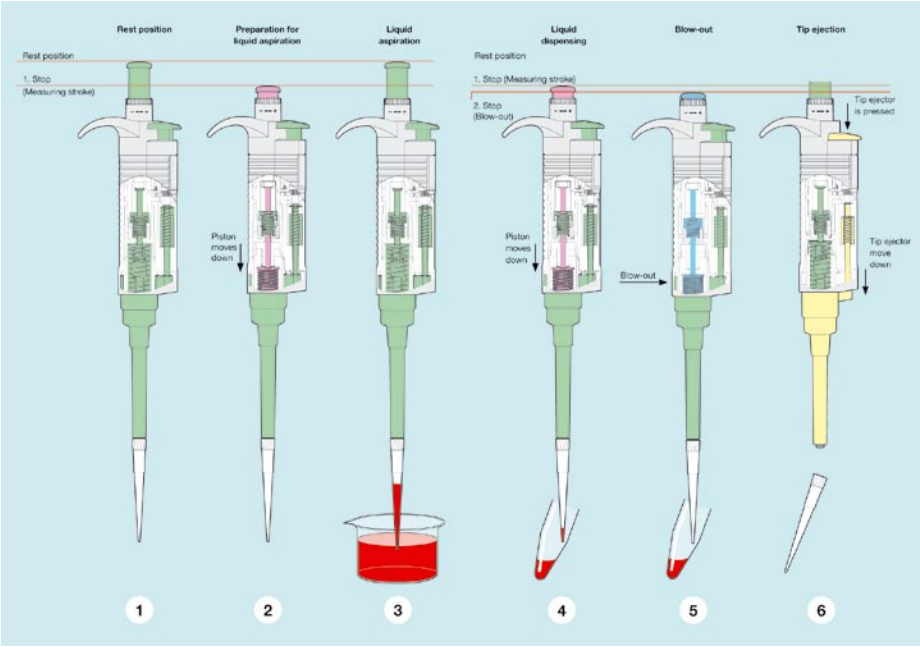

Fig. 2: Function of an air-cushion pipette (Eppendorf Research)

ed piston, which is coupled to the piston rod of the dispensing device during the pipetting (Fig. 3) and the actual dispensing process. The tips are specially designed for the use of positive-displacement systems and cannot be replaced by tips foreign to the system.

Figure 4 shows the functioning of positive displacement. To prepare for aspiration of the liquid (1), the push-button is pressed to the first stop and the piston moves down to the corresponding position. For aspiration of the liquid (2), the pipette tip is immersed a few millimeters into the liquid vertically, the push-button is then allowed to slide back slowly, the piston moves up, and the required volume of liquid is aspirated into the tip by the partial vacuum that is produced. To dispense the liquid into a vessel (3), the push-button is slowly pressed to the first stop (measuring stroke). The piston in the tip is moved down by the piston rod of the pipette, thus displacing the liquid from the tip. The push-button is held down, and the tip drawn up against the wall of the vessel. To eject the tip (4), the push-button is pressed all the way down.

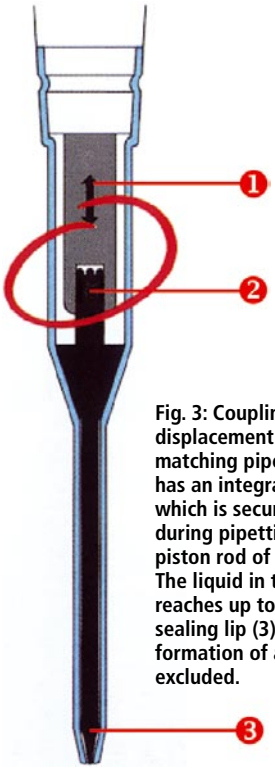

Fig. 3: Coupling of a positive-displacement pipette with a matching pipette tip: the tip has an integrated piston (2) which is securely connected during pipetting with the piston rod of the pipette (1). The liquid in the tip only reaches up to the hermetic sealing lip (3), thus the formation of aerosols is excluded.

Overview of Dispensing Systems (Fig. 5)

Lab devices for the transport and preparation of liquid samples can be divided into single-function dispensing systems (manual piston stroke pipettes and dispensers) and multifunction dispensers (electronic pipettes and dispensers, auto diluters). The demands made on the device technology mainly depend on the application. The spectrum ranging from routine to research applications reflects the extent of the requirement profile made on dispensing technique today. See below for a description of the current status of dispensing systems.

Piston-stroke Pipettes

Piston-stroke pipettes are designed for the dispensing of fixed or adjustable volumes in a range from below 0.1 µl to 10 ml. They function either according to the air-cushion principle or the positive displacement principle.

High-quality piston stroke pipettes of the new generation are characterised by compact and robust design as well as maximum operational comfort. The pipette pistons are resistant to chemicals and corrosion-proof. As the piston-stroke pipettes are either fully or partially autoclavable, any residual doubts regarding sterility are unnecessary, thus opening up new fields of application for these products. The plastics used to make these pipettes are UV-resistant; this is of central importance for many applications. This means that UV-resistant pipettes can be left without risk in inoculation rooms or on benches as the ultraviolet light used to disinfect workstations does not have any adverse effect on the pipette material and thus on the functionality of the pipette. Piston-stroke pipettes can generally be adjusted to dispense liquids with a density different to that of water.

Single Channel and Multichannel Pipettes

With single channel pipettes a distinction is made between fixed-volume pipettes (e.g. 100 µl, 500 µl or 1,000 µl) and variable, or adjustable-volume pipettes covering a coherent volume range (e.g. from 0.5 to 10 µl or from 500 to 2,500 µl).

Multichannel pipettes are designed for use with microtest plates. With their channels generally numbering eight or twelve they greatly reduce the amount of necessary pipetting processes, resulting in a major time saving with great comfort. Multichannel pipettes function according to the air-cushion principle.

Pipette Tips

To meet the growing demands on reliability and reproducibility as well as the trend towards smaller sample volumes dispensing devices with an ever-increasing accuracy are required. Disposables reduce both the operational effort and the risk of contamination when handling infectious or radioactive liquids.

The pipette tip is a key component of the „pipette" dispensing system. Its shape, material properties and fit have a major impact on the accuracy of the dispensing process. It is only possible to achieve the maximum precision and reliability offered by modern pipettes with perfectly manufactured pipette tips and optimum coordination between the pipette and tip.

Pipette tips must be precisely shaped to ensure pipetting accuracy in the microliter range. They must be designed so that even the tiniest drops can be dispensed accurately on the surfaces of micro test tubes. If these requirements are not fulfilled, errors will already occur during the preparation of samples that are often far more serious than errors directly associated with the analysis procedures.

Dispensers

Dispensers are also used to reduce the number of individual pipetting processes so ergonomics

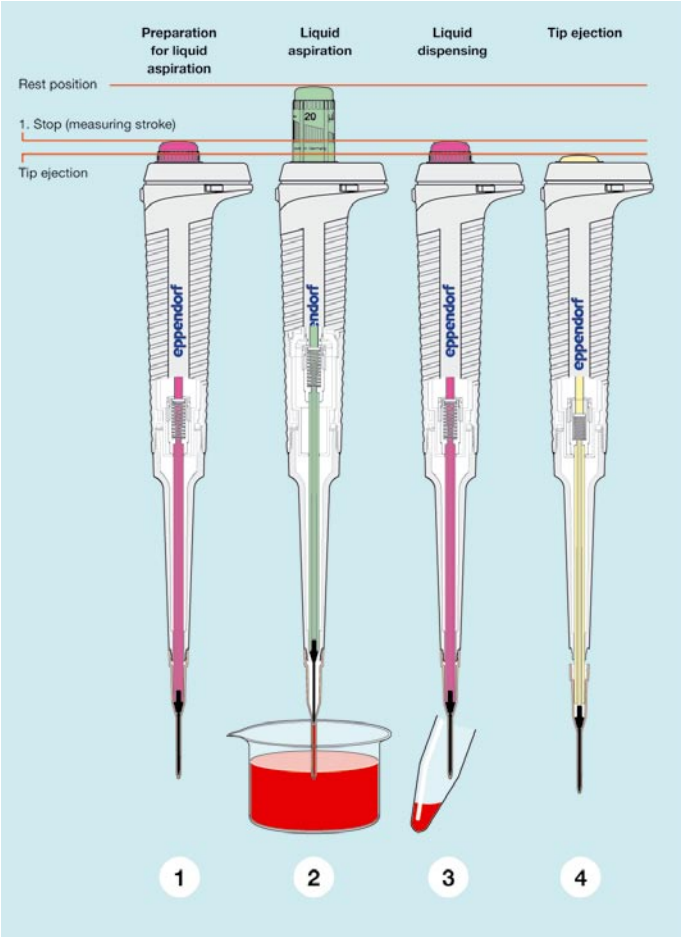

Fig. 4: Function of a positive-displacement pipette (Eppendorf Biomaster)

plays a key role here too. A certain volume is repeatedly dispensed from a previously aspirated volume. A precise number of repeated liquid dispensing processes can be carried out from the reservoir, depending on the selected setting of the dispenser. Dispensing of a single volume into a large number of vessels and plates using a handheld dispenser is the preferred option if automation is not possible or would be too costly.

Dispensers enable faster processing of long test series with a high dispensing accuracy and provide great flexibility with regards to applications and sample volumes. They always operate according to the principle of positive displacement. Every press of the thumb on the pipetting lever results in a mechanical stepped feed which is transferred to the piston of the syringe-like plastic tip. A selection of cylinder sizes offers a large number of different sample volumes ranging from 1 µl to 10 ml. Modern dispensers reduce user error by displaying the pre-selected dispensing volume on an integrated display.

Applications in the field of chemistry often involve the dispensing of acids, bases, solvents and saline solutions whose density, vapour pressure and viscosity differ widely from the corresponding values for water. As dispensers function according to the positive displacement principle, they offer high levels of dispensing accuracy comparable to the values for water for such substances. They greatly depend on the reliability of the plastic tip, whose piston and cylinder consist of two different materials – polypropylene (PP) and/or polyethylene (PE).

Compared to the air-cushion principle, the flow properties and the wetting behavior of the liquids to be dispensed are of lesser importance with positive displacement systems.

Electronic Dispensing Systems

Electronic pipettes and dispensers are semi-automatic systems and

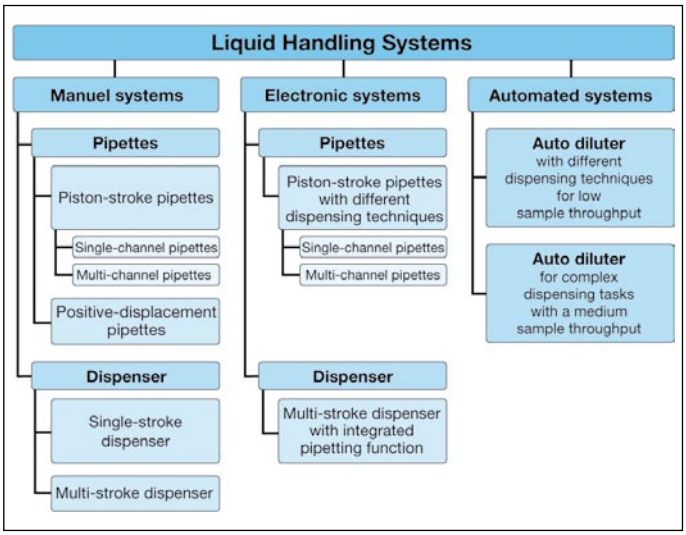

Fig. 5: Liquid handling systems in the lab

are suitable for a variety of applications besides standard pipetting. These systems fall between conventional manual pipettes and dispensers on the one hand and fully automatic systems including pipetting robots on the other hand.

The great advantages of electronic pipettes are their high reproducibility, reduced pipetting forces and the multifunctionality, which opens up a wide field of application. Thanks to adjustable and constant dispensing speeds the results can be far more accurate than with manual pipettes. Aspiration and dispensing of the liquid is initiated at the touch of a button, thus saving both time and effort. The multifunctionality resulting from various dispensing techniques such as simple dispensing, reverse pipetting, diluting and mixing makes the work process much easier. All these functions can be programmed individually and stored in the pipette's memory for additional time savings when performing routine procedures.

Key features are user-friendliness and ergonomics to reduce fatigue from the constant repetition of movements and to so avoid resulting risks to health.

Automated Systems

An automated liquid handling system generally consists of a robotic arm (XYZ transmission system for positioning) and a dispensing device. Such systems provide speedy and reproducible dispensing via pipetting, dispensing or other dispensing processes. Processes which have been previously carried out manually can be quickly and easily transferred to automatic systems. As the sequence of movement is freely programmable, it can be used for a whole range of tasks.

Outlook

Two trends in particular have emerged in the field of life sciences in recent years: firstly, a reduction in sample volumes and secondly, an increase in the number of samples processed. The standardised microtest plates with 96 or 384 wells used in the lab make the precise and error-free manual dispensing of liquids into the wells almost impossible for the user. The distance between the wells of a 384-well plate is for example only 4.5 mm. It is only the automation of such processes that makes it possible to reliably carry out a high number of dispensing steps with low sample volumes.

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