Exact and reproducible dispensing of liquids is a prerequisite for many applications in medical and molecular biology laboratories. The performance of test systems is largely dependent upon the exact proportions of the individual reaction components in an assay. Modern pipettes enable the user to manually dispense amounts of liquid down to about 0.1 µL. Using this mature technology, even users with little experience can dispense such small amounts of liquid without fearing the effects on the precision of the results.

Despite the great importance of precise liquid handling in research and diagnostics, little attention is often paid to the hardware responsible for it, namely the pipettes.

Here, I will give an overview of the technical principles of pipettes and discuss the characteristics of different systems defined by different methods of construction. I will also classify them by their applications. Other factors that affect the precision and accuracy of dispensing liquids will also be examined. Some practical tips will be given outlining even more precise methods of working.

Construction of Piston-Stroke Pipettes

Conventional glass pipettes are characterized by their simple mode of construction, but they are difficult to handle and the precision of the pipetting results is very dependent upon the ability of the user. Modern piston-stroke pipettes are more complex, but are very straightforward and easy to use. A piston-stroke pipette has the following construction characteristics: liquid is measured using a piston-cylinder system in the handle of the pipette. The liquid, in contrast, is taken up into a disposable pipette tip or dispensed out of it. A pipette can be regarded as a system, consisting of a pipette and the tip, whose precision can only be judged as a whole system.

Two construction principles have found success: the air cushion principle and the positive displacement principle. In the air cushion principle, an air cushion separates the liquid in the tip from the piston inside the pipette. The piston moves the air cushion and the liquid is thus taken up into the pipette tip or dispensed out of it. The air cushion works like an elastic spring, to which the liquid sticks. Since the air space is stretched during pipette aspiration, the pipette must move a volume about 2 to 4% greater than the aspirated volume of liquid. The manufacturer must take this fact into consideration during construction of a pipette. [F1].

In the positive displacement principle, a pipette tip constructed like a syringe is used. The pipette tip thus contains its own piston, which is operated by the pipette mechanism. Because of this unique mechanism, positive displace-
Electronic pipettes are constructed as either positive displacement or air cushion systems. Since a motor carries out the piston movement, control is independent of the user’s finger movement. Even inexperienced or tired users are able to pipette automatically at the same speed. The reproducibility of results is thus always guaranteed [F2].

**Precise Pipetting**

Air cushion pipettes can deal with a large proportion of laboratory applications. Any aqueous solutions can be dispensed with instrument-specific precision and accuracy. However, if liquids with very high densities (eg glycerine) or very high vapor pressures (eg chloroform or hexane) are used, the precision may suffer. If this is not realized, then, for instance with chloroform, the pipette leaks and will generally dispense too little liquid. This occurs partly because some of the liquid evaporates and the liquid leaks out of the tip. Naturally, the viscosity and surface tension of a liquid also play a role. If the tip of the piston-stroke pipette is prewetted several times previously, the air cushion may be partly saturated with chloroform, and, thus, the precision of the pipetting procedure may be improved. Direct displacement systems, in which factors that affect properties of an air cushion play hardly any role, are more suitable for such purposes. Any small air bubble forming in the direct displacement tip is easily saturated with vapor because of the small volume. Highly accurate pipetting with exact drip separation is then possible.

Liquids with extremely high density represent another extreme situation. With air cushion pipettes, the extent to which the air volume expands depends on the density of the liquid being pipetted. In other words, if the liquid is heavier than water, the air cushion is stretched more than with an aqueous liquid. With a liquid that is heavier than water, too little will be taken up, and, thus, too small a volume will be pipetted. For instance, if a liquid with a density of 1.1 mg/µL is pipetted, the error is in the order of 0.2%. This error can be remedied by readjusting the pipette.2

In this case, it is also advantageous to use a positive displacement system, in which air cushion-dependent phenomena do not play a role.

### Other Hints for Optimal Pipetting Technique

Several physical parameters affect the accuracy of pipetting and allow optimal pipetting techniques to be identified. Aspirating and dispensing of liquid using air cushion pipettes should be performed using the same hydrostatic pressure in order to achieve exact pipetting results. For this reason, the pipette tip of an air cushion pipette should be placed only a few millimeters below the surface during aspiration. The pipette should also be held almost vertical. If the pipette is held on the slant, the height of the liquid column in the tip will fall, and more liquid will be taken up — an error in the volume will result. If the pipette is held at an angle of 30°, up to 0.7 % more liquid can be taken up.

Optimal drop separation from the pipette tip is largely determined by the quality of the pipette tips used. The contour of the pipette tip is crucial. Irregularities, which can arise during the tip production, such as edges or excess length, markedly affect the flow properties of the sample liquid from the tip and thus the results. To optimize the flow behavior of the liquid from the pipette tip, the pipette tip should be placed against the wall of the micro test tube during the dispensing of the liquid.

Another factor is the interaction between the liquid and surface of the tip. During pipetting, part of the liquid remains as a thin film in the pipette tip.

<table>
<thead>
<tr>
<th>Predominant type of error</th>
<th>a and b</th>
<th>a</th>
<th>b</th>
<th>—</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) random errors</td>
<td><img src="chart1.png" alt="random errors" /></td>
<td><img src="chart2.png" alt="random errors" /></td>
<td><img src="chart3.png" alt="random errors" /></td>
<td><img src="chart4.png" alt="random errors" /></td>
</tr>
<tr>
<td>b) systematic errors</td>
<td><img src="chart5.png" alt="systematic errors" /></td>
<td><img src="chart6.png" alt="systematic errors" /></td>
<td><img src="chart7.png" alt="systematic errors" /></td>
<td><img src="chart8.png" alt="systematic errors" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Precision</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>bad</td>
<td>bad</td>
</tr>
<tr>
<td>bad</td>
<td>good</td>
</tr>
<tr>
<td>very good</td>
<td>bad</td>
</tr>
<tr>
<td>good</td>
<td>good</td>
</tr>
</tbody>
</table>

[F3] Precision and accuracy of a pipette illustrated by a coordinate system.
For aqueous liquids, this is taken into account in the construction of the pipette. In case of samples that require a lot of wetting, this possible error can be minimized by prewetting the tip several times.

**Pipette Calibration**

Pipettes are testing equipment as defined by GLP guidelines and comparable quality assurance norms (ISO 9000, EN 45000). It is crucially important to know how accurately the pipettes work.

**Accuracy of Pipettes**

The evaluation is based on two parameters. Figure 3 makes it clear that in pipetting random and systematic errors come into play. We therefore describe the pipette in terms of its accuracy and precision [F4].

Calibration of pipettes means testing the precision and accuracy of a pipette. Adjustment of a pipette on the contrary, refers to changing the setting of a pipette if calibration shows that precision or accuracy of the system are out of range.

Another calibration is necessary to verify the results of an adjustment.

The gravimetric method is usually used to test a pipette. Distilled water is pipetted and weighed on an analytical balance. The mass \( m \) of the pipetted water and its density \( p \) at the current room and water temperature are used to calculate the volume \( V \): \( V = \frac{m}{p} \).

**Requirements**

For pipette calibration, you need an analytic balance and evaporation trap to
minimize evaporation of the test liquid. The temperature of the test liquid (distilled water) and of the room must also be kept constant (between 20°C and 25°C ± 0.5 °C), since the density of water is temperature dependent [F4].

According to DIN 12650 it is necessary to perform 10 measurements of the nominal volume of a fixed-volume pipette to evaluate precision and accuracy. An extensive description of the procedure for pipette calibration can be found in the standard operating procedure for pipettes from Eppendorf AG.3

Modern calibration software, such as PICASO from Eppendorf, enables easy and rapid evaluation of gravimetric measurements, with the analytical scale directly linked to the computer. Test data can be handled easily and the relevant test protocols are produced.

Laboratories that do not want to undertake their own calibration can get authorized dealers to calibrate the pipettes and obtain a test sheet with precise data.

The Future

Eppendorf has largely fuelled the microliter system in introducing the first industrial piston-stroke pipette in 1961, as well as by inventing the legendary Eppendorf micro test tube. Now, an attempt to reach even smaller dimensions is planned, namely the introduction of a Nanozyme dispenser, which enables precise manual dispensing of liquid volumes between 10 nL and 1 µL.3 As test analyses undergo further miniaturization, characterized by reaction vessels such as 384- and 1,536-well microtiter plates and chips, nanoliter dispensing will gain central importance.