

# Impact of pipetting techniques on precision and accuracy

Kornelia Ewald, Eppendorf AG, Hamburg, Germany

## Introduction

The fields of application for dispensing systems depend on the design of the system as well as the physical limits inherent to the application. To fulfill the high requirements in terms of precision and accuracy the application

primarily calls for a precise device with high-quality tips and also, sufficient practical experience, correct handling and cleanliness of the dispensing systems.

## Dispensing techniques

The dispensing techniques used most frequently are forward pipetting, reverse pipetting, dispensing, sequential dispensing and diluting. While mechanical pipettes are only suitable for forward and reverse pipetting, electronic dispensing systems frequently cover all of the above functions. Selection of the dispensing technique suitable in each case may have a significant impact on the analysis results.

The technique of forward pipetting (Fig. 1) is suitable for aqueous solutions which may contain low concentrations of protein or detergents. Pre-wetting of the tip improves the analysis result.

With viscous or foaming liquids or when dispensing very small sample volumes the results may be greatly improved by reverse pipetting. Here the liquid is aspirated with blow-out and dispensed without blow-out. A residue of liquid remains in the plastic tip, and it is subsequently discarded or returned to the aspirating vessel (Fig. 2).

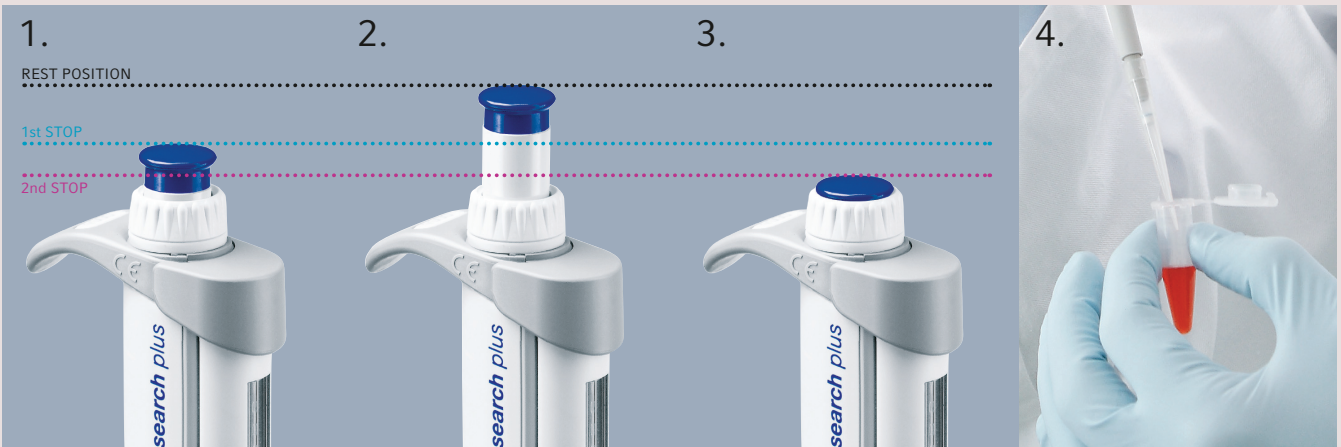
During the process of dispensing the aspirated liquid is dispensed in defined steps. This technique is often used when processing long test series or filling microtest plates.

With sequential dispensing, different volumes of a solution are dispensed one after the other in a specified sequence. This technique is often used with serologic work processes and similar applications.

When diluting liquids an initial volume is first aspirated, followed by an air bubble and the second volume. Both volumes are then dispensed in one step. This dispensing technique increases throughput and reduces fatigue compared with performing aspiration and dispensing twice with manual pipettes – particularly in the case of sizeable volumes and multichannel pipettes.

## Forward and reverse pipetting

Transfer	Forward pipetting
<b>Liquid uptake</b>	1. Press operating button down to 1st stop 2. Let operating button move up completely
<b>Liquid discharge</b>	3. Press operating button via 1st stop down to 2nd stop
<b>Observation</b>	4. No liquid is left in tip after action



## Reverse pipetting

1. Press operating button down to 2nd stop
2. Let operating button move up completely
3. Press operating button down to 1st stop
4. Liquid is left in tip after action (volume of blow-out)



## Optimum handling of manual pipettes

Irrespective of the dispensing technique used, the following items should be taken into consideration during pipetting:  
 | In the case of air-cushion pipettes, the pipette tip should be selected so that the air cushion between the pipette piston and the surface of the liquid is as small as possible. The smaller the tip, the lower the air volume, and the greater the accuracy of the results will be.

- > When aspirating the liquid, the tip should only be immersed a few millimeters into the medium (Tab.1, Fig. 3).
- > The filled tip should be moved up against the wall of the vessel to avoid residues of liquid on the outside of the tip.
- > Pre-wetting the tip two or three times will improve the accuracy and precision of the results.
- > Liquid should be aspirated slowly and evenly.
- > A waiting period of 1 to 3 seconds should be allowed for the liquid to rise in the tip.

The pipette should be held vertically during aspiration. On aspiration of the liquid the hydrostatic pressure of the liquid column in the tip falls as the angle of inclination of the pipette increases. This will result in an increased aspiration volume [1]. The deviation in volume can be estimated for any pipette volume and angles of inclination. With an angle of 45° diverging from the vertical the extra volume with a 1000 µl pipette is 2.9 µl or 0.29 %. For a 100 µl pipette at an angle of 60°, an extra volume of 0.53 µl or 0.53 % will result (Fig. 3).

The fact that different people do not pipette exactly the same volume with exactly the same pipette can also be explained by factors such as different angles of inclination when aspirating liquid. Although the effect is small in each instance, the error may be much greater when all of the differing factors are added together.

Table 1

Volume (µl)	Optimum immersion depth (mm)
0,1 - 1	1
1 - 100	2 - 3
101 - 1000	2 - 4
1001 - 10000	3 - 6

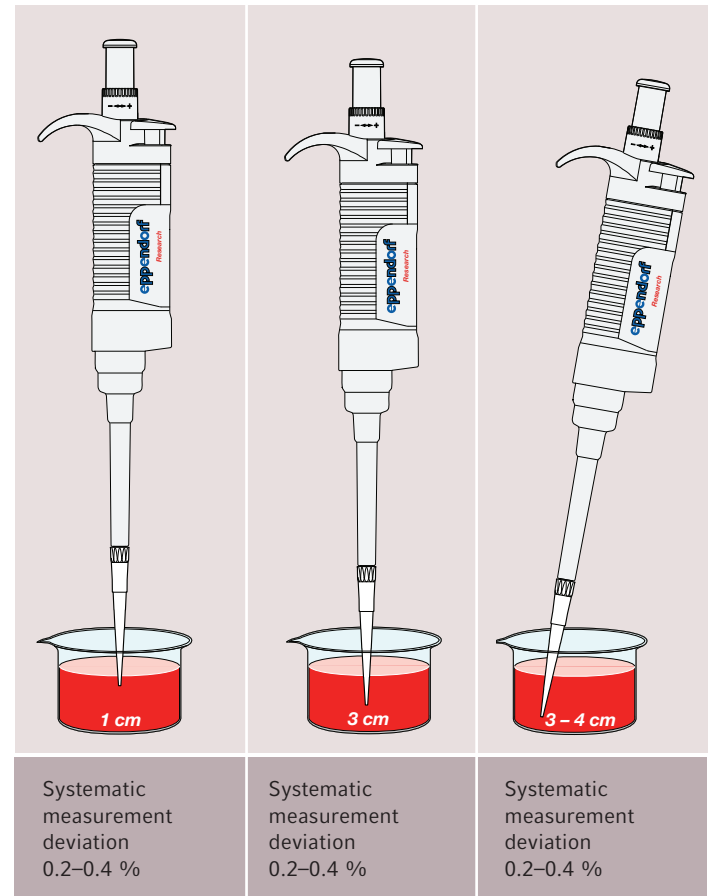


Figure 3:

Influence of immersion depth and holding angle of pipette during aspiration of liquid:

1. Pipette vertical, immersion depth of tip in liquid approx. 1 cm
2. Pipette vertical, immersion depth of tip in liquid approx. 3 cm
3. Pipette at a holding angle of 30° to 40°, immersion depth of tip in liquid approx. 3 cm to 4 cm

## References

- [1] Lochner, K. H.; Ballweg, T.; Fahrenkrog, H.-H.: Untersuchungen zur Messgenauigkeit von Kolbenhubpipetten mit Luftpolster. In: Lab Med 20 (1996), Nr. 7/8, S. 430–440.

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