

## Pipetting

# Dispense liquids containing proteins more reliably with reverse pipetting

This application note demonstrates how the reverse pipetting technique can improve the repeatability of dispensing liquids that contain proteins.

### Introduction

A pipette's volume is commonly adjusted using pure water and the forward pipetting technique. This technique is recommended for aqueous solutions, such as buffers, diluted acids or alkalis. When pipetting liquids other than water, the dispensed volume may deviate from the selected one due to varying liquid properties (Koivisto, 2007). Some liquids, including biological solutions, may cause bubbles or foam to form in the pipette tip or test tube, which can result in volume deviations.

The reverse technique is recommended for pipetting solutions with high viscosity or a tendency to foam. Reverse pipetting reduces the risk of splashing, foam or bubble formation. This method is most beneficial when dispensing small volumes.

### Forward Pipetting

1. Press the operating button to the first stop.
2. Immerse the tip into the solution to a depth of 1 cm and slowly release the operating button. This action fills the tip. Withdraw the tip from the liquid, touching it against the edge of the reservoir to remove excess liquid.
3. Dispense the liquid into the receiving vessel by gently pressing the operating button to the first stop. After one second, press the operating button to the second stop. This action empties the tip. Remove the tip from the vessel, sliding it along the wall of the vessel.
4. Release the operating button to the ready position.  
Reverse Pipetting
5. Press the operating button to the second stop.
6. Immerse the tip into the solution to a depth of 1 cm and slowly release the operating button. This action fills the tip. Withdraw the tip from the liquid, touching it against the edge of the reservoir to remove excess liquid.



7. Dispense the liquid into the receiving vessel by depressing the operating button gently and steadily to the first stop. Hold the button in this position. Some liquid will remain in the tip, and this should not be dispensed.
8. The liquid remaining in the tip can be pipetted back into the original solution or thrown away with the tip.
9. Release the operating button to the ready position.

**Calibration** = determination of the difference between the mean volume of the measurement series and the selected volume on the pipette display.

**Adjustment** = alteration of the pipette settings so the actual volume corresponds to the selected volume.

**Inaccuracy** = (systematic error) the difference between the dispensed volume and the selected volume of a pipette.

**Imprecision** = (random error) the repeatability of the pipettings.

## Materials and methods

**Test pipette:** Thermo Scientific™ Finnpiquette™ F2 pipette 1-10 µl

**Test tips:** Thermo Scientific™ Finntip™ Flex™ 10 pipette tip

**Test liquid:** 1% bovine serum albumin (BSA, Sigma A7030)

Five series of ten pipettings each were performed using both the forward pipetting technique and the reverse pipetting technique. The test volumes were 1 µl and 10 µl, and the pipette was adjusted for each technique.

## Results

The results of pipetting 1 µl 1% BSA using the forward and the reverse pipetting technique are displayed in Figure 1. The figure shows that when the reverse pipetting technique was used, the dispensed volume variations were within a narrower range than when the forward pipetting technique was used.

Figure 2 shows the degree of imprecision using each pipetting technique. Imprecision is a measure of the repeatability of the pipettings. With the reverse pipetting technique, the imprecision was reduced by over 50% as compared to the forward pipetting technique.

## Discussion

The pipette tip material is hydrophobic to ensure the best removal of aqueous solutions.

A BSA solution contains hydrophobic components that tend to be attracted to the hydrophobic pipette tip wall. When using the forward technique, a small amount of liquid tends to remain inside the tip after each pipetting step. This tendency increases the deviation between dispensed liquid volumes because the accumulated extra volume may increase the next dose when pipetting is repeated.

With the reverse pipetting technique, an excess of liquid is taken up into the tip. This excess functions as a reservoir that evens out the sequential volumes. The reservoir also prevents air from passing through the orifice at the end of the dispensing step, which reduces the possibility of foam formation. This makes the reverse pipetting technique especially useful when delivering small volumes.

In this study the test pipettes were adjusted for water, which is a recommended standard procedure (e.g. ISO 8655). Due to its different liquid properties, the inaccuracy results with the BSA solution deviated from the results obtained with water. The pipetting technique also had an effect on the inaccuracy value.

The difference to the adjustment value obtained with water was determined by calibrating the pipettes with the liquid used in the application. The observed difference was compensated by changing the dispensed volume.

## Conclusion

The above-mentioned phenomena demonstrate that greater precision dispensing small volumes of liquids containing proteins can be achieved by using the reverse pipetting technique. This is good to keep in mind when pipetting small volumes of other biological solutions or liquids that have a tendency to foam.

## References

1. Koivisto S. 2007. The tip of perfection – Factors affecting pipetting performance. Laboratory Equipment 8, pp 38-40.

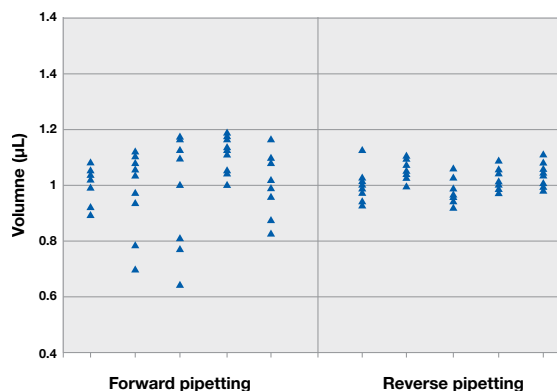


Figure 1. Pipetting 1 µl 1% BSA using the forward and reverse pipetting techniques

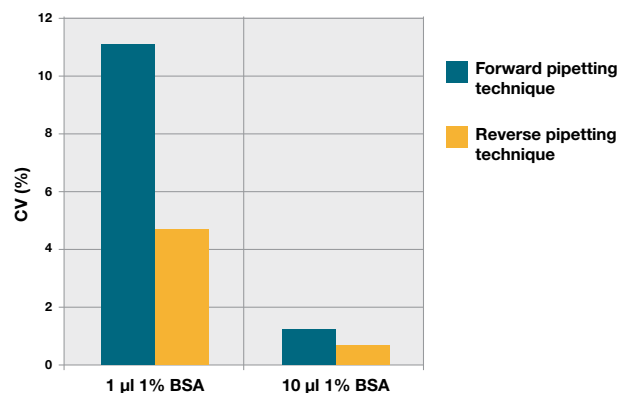


Figure 2. Imprecision of pipetting 1% BSA solution with different techniques