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How to Handle Challenging Liquids with the epMotion® 96 Flex

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Executive Summary

Using a 96-channel semi-electronic pipette like the epMotion® 96 Flex for handling challenging liquids such as viscous, dense, volatile and protein-rich reagents or detergents needs special techniques and considerations to obtain best accuracy and precision. Liquids other than water influence the air cushion in the pipette tip between sample and piston in different ways. In this white paper we give recommendations and handling advices for commonly used liquid types in the laboratory. Following these tips and tricks you will be able to improve your handling of challenging liquids and obtain more accurate, reliable and reproducible results.

Introduction

A huge variety of applications exists in laboratories worldwide. Each research segment has different demands in terms of sterility, volume range, plates and tubes, final read-out method, etc. But some things all laboratories have in common: the usage of liquid handling tools such as pipettes or dispensers and the handling of different challenging liquids with different physical properties than water. Among these viscous solutions such as glycerol and DMSO are very common, but also volatile liquids such as acetone or detergents are widespread. Some liquids might tend to form foam and bubbles due to their high protein content. The difficulties in pipetting these liquids arise in one type of pipette, the air-cushion pipettes. This type of pipette is most common and used in almost every lab worldwide. The air cushion exists between the sample liquid in the tip and the piston inside the pipette. During normal operation with aqueous solutions like water or standard buffers (e.g. PBS, Tris-HCl) the air is first exhaled by a downwards piston movement to create a partial vacuum which then allows liquid aspiration. By the following downward piston movement the remaining air in the pipette is compressed and the liquid is dispensed. The air in the pipette acts like an elastic spring and is moved up and down in each pipetting stroke. As mentioned before, this technique is ideal for water and aqueous solutions such as buffer and low-salt solutions. But if a challenging liquid is pipetted the air cushion in the pipette reacts differently. Depending on the liquid type this can lead to inappropriate volume delivery, liquid dripping or foam formation [1]. The most common liquid types and impacts are discussed further before giving tips for correct handling with the epMotion® 96 Flex.

Viscous and dense liquids

Common viscous and /or dense liquids in the lab are glycerol, DMSO or liquid collagen. These liquids are used for sample storage or preparation of 3D cell models in microbiology and cell culture. The viscosity is caused by a high inner friction of molecules leading to bad flow behavior. Pipetting a viscous or dense liquid leads to inappropriate liquid intake because the drag force of the partial vacuum inside the pipette does not suffice to aspirate the correct amount of liquid. Another problem occurring with viscous or dense liquids is insufficient liquid dispensing because pushing down the operation button provokes the air between the viscous sample and the pipette piston to compress further. Due to the high density of viscous liquids these are not pushed out properly by the air and remaining liquid will stay inside the tip. This leads to an inaccurate pipetting result.

Volatile liquids

Working with a volatile liquid such as acetone, ethanol or chloroform is daily routine in most laboratories, e.g. for DNA extraction and purification. Volatile liquids have a high vapor pressure and the air-cushion inside the pipette expands when these liquids evaporate into the pipette tip and pipette cone. This leads to immediate dripping of the liquid out of the pipette tip. Possible outcomes are inaccurate pipetting results, sample loss and drops of chemicals on the bench.

Detergents

Detergents are mainly used in buffers to reduce the surface tension of water. Common detergents are Tween® 20 or Triton™ X-100. These liquids show a slow flow behavior and aspiration takes a lot of time. Furthermore detergents often have a higher density than aqueous solutions which leads to compression of the air-cushion while dispensing. But the main problem is sticking of detergents to the inside of the pipette tip. A thin layer of liquid always remains inside the tip and only flows down very slowly. The compressed air inside air-cushion pipettes cannot dispense this remaining liquid. This leads to sample loss and inaccurate pipetting results.

Protein-rich liquids

Examples for solutions containing high protein amounts are buffers containing bovine serum albumin (BSA) or cell culture medium. Protein-rich solutions can lead to foam formation because proteins are surface active substances of high molecular weight. These can enrich at the liquid-gas interface and lead to gas bubbles covered in liquid, called foam. Foam built of proteins is extremely stable and difficult to remove therefore avoiding it is the better strategy. During pipetting the liquid is aspirated and dispensed, so the surface where proteins enrich is constantly changing. When reusing a pipette tip, already a thin layer of liquid with contact to air is present inside the tip. Liquid that is aspirated touches and swirls this interphase leading to foam formation inside the tip. Additionally performing the blow-out introduces air bubbles into the sample. Each air bubble has a liquid-air interface stabilizing the foam. Omitting the blow-out leads to inaccurate volume delivery. Furthermore foam can disturb cell growth or final read-out methods such as photometric measurements.

Solutions & Benefits

1. Tip immersion
Immerse the tip not more than 3 mm for 50 µL and 6 mm for 300 µL and 1,000 µL tips into the liquid.

2. Pre-wetting
To saturate the air in the pipette tip pre-wet 2-3 times using the pipette & mix function at a low speed level of 2-4 before switching to the desired mode. Use the same amount of volume for mixing as you want to use for the desired volume transfer.

3. Liquid aspiration
Aspirate liquid with the tips immersed into the liquid. When aspiration is finished wait until the liquid level stops ascending in the tip.

4. Liquid dispensing
Dispense the liquid with the tips in contact to the liquid surface or if the plate is empty slightly over the plate bottom. For small volumes < 5 µL dispensing into liquid is essential.

5. Blow-out
Perform the blow-out step with the tips immersed into the liquid. In some modes the blow-out has to be discarded: multidispense, reverse pipette and small volume.

6. Reverse pipetting
Blow-out is performed prior to liquid aspiration leading to aspiration of the set volume plus the additional blow-out volume. After dispensing the blow-out is not performed so that some liquid remains inside the tip.

Table 1 summarizes handling advices for each liquid type discussed in this white paper. Additionally we recommend the operation mode that is most beneficial for mastering the various challenges. With these tips and tricks the epMotion® 96 Flex will help to increase pipetting accuracy and precision with commonly used challenging liquids.

Table 1: Tips for successful transfer of challenging liquids wit the epMotion® 96 Flex

Liquid examples	Liquid type (properties)	Operation mode	Tips for successful liquid transfer
Aqueous solutions	Water-like properties	All modes	> Follow the general pipetting practices for epMotion® 96 Flex
Ethanol Methanol Acetone Chloroform	Volatile	Reverse pipette	> Speed level 3 to 6 > Pre-wet the tips with the liquid at least 5 times > Perform the liquid transfer rapidly > Avoid long elapsed time between aspiration and dispensing
Glycerol 30-85 %	Viscous	Reverse pipette	> Speed level 1 to 3 > Tips must remain in the solution until all liquid is aspirated or dispensed > Use of the epMotion® 96 Flex for highly viscous liquids such as 99 % glycerol is not recommended. A positive displacement pipetting system such as a Multipette®/Repeater® is a better option.
Tween 20 0.1-1 %	Detergent	Reverse pipette	> Follow the general pipetting practices for epMotion® 96 Flex
		Multidispense	> No blow-out when re-using the tips
		Pipette	> Speed level 1 to 3 for concentration > 1 % > Tip exchange after each dispensing step > No re-use of tips > Increase time for liquid dispensing before performing blow-out
BSA 1 % Cell culture medium	Protein-rich solution	Reverse pipette	> Follow the general pipetting practices for epMotion® 96 Flex
		Multidispense	> Do not re-use tips > No blow-out when re-using the tips > Ensure tip is only immersed to 1-2 mm below the liquid surface
DMSO 1-10 %	Dense and viscous	Reverse pipette	> Follow the general pipetting practices for epMotion® 96 Flex
DMSO 90 %	Dense and viscous	Reverse pipette	> Speed level 1 to 3 > Tips must remain in the solution until all liquid is aspirated or dispensed > Use of the epMotion® 96 Flex for highly viscous liquids such as 90 % DMSO is not recommended. A positive displacement pipetting system such as a Multipette®/Repeater® is a better option.

References

[1] The science of pipetting perfection. A guide to expert pipetting. www.eppendorf.com/pipetting-ebook

Ordering information

Description	Order no. international
epMotion® 96 Flex base device, without dispensing head, with 2-position slider, including TipHolder for epMotion 96 Flex for use of epTIPS Motion Reloads, 100 - 240 V, 50 - 60 Hz.	5069000519
Dispensing Head 96-channel 0.5 - 300 µL, for epMotion 96 Flex in hard shell case.	5069000527
Dispensing Head 96-channel 5 - 1000 µL, for epMotion 96 Flex in hard shell case.	5069000535
TipHolder for epMotion 96 Flex	5069075004

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