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WHITE PAPER No. 39

How to Handle Problematic Liquids with the ep*Motion*® 96 and ep*Motion*® 96xl

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Executive Summary

Using a 96-channel air-cushion pipettor like the ep*Motion*® 96 and ep*Motion* 96xl for pipetting challenging liquids such as viscous, dense, volatile and protein-rich liquids or detergents needs special techniques and considerations to obtain best accuracy and precision. Liquids other than water influence the air cushion between sample and piston in different ways. In this white paper the reactions of the air cushion for most common challenging liquids are explained and handling advices for the ep*Motion* 96 pipettors are given. These include recommendations on the mode to use and the technique that should be applied. Viscous liquids should be pipetted in reverse mode and with a low speed while for volatile liquids pre-wetting is essential. Detergents and protein-rich liquids should be pipetted in reverse pipette mode avoiding the blow-out and without reusing the tips. Following these recommendations you will be able to improve your handling of problematic 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 almost everyone is using problematic liquids and solutions. Problematic liquids are defined as liquids with other properties than water. Among these viscous solutions such as glycerol are very common, but also volatile liquids such as acetone or detergents (Tween® 20) are widespread. Some liquids might tend to form foam and bubbles due to the 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. Air is present between the sample 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, it 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 in the pipette reacts differently. Depending on the liquid type unwanted reactions of the air-cushion can lead to inappropriate volume delivery, liquid dripping or foam formation. The most common liquid types and impacts are discussed further before giving tips for correct handling of these liquids.

Viscous and dense liquids

Common viscous and /or dense liquids in the lab are glycerol 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 during DNA extraction and purification. These liquids confront the pipette with a huge challenge. 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 solutions

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 [1]. 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.

Pipetting these liquids with manual single-channel pipettes is already a challenge. Using eight to twelve-channel pipettes or even 96-channel pipettors like the ep*Motion* 96 and ep*Motion* 96xl increase the challenge even more (Fig.1). Therefore correct handling of problematic liquids with pipettes is essential to obtain high accuracy and precision. Table 1 lists common difficult example liquids and explains how to handle the liquids to obtain the best pipetting result possible.

Figure 1: The ep*Motion* 96 is a 96-channel pipettor that works according to the air-cushion principle and is mainly used for plate work.

Solutions & Benefits

Pipetting modest solutions such as water, buffer or salt solutions is daily routine. Some general pipetting practices with the ep*Motion* 96 and ep*Motion* 96xl should be considered anyway. These pipetting practices lead to high accuracy and precision when handling aqueous solutions.

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.

When it comes to dealing with problematic liquids either special tools or pipetting techniques are required. When using a 96-channel pipettor extra caution is demanded because difficulties showing once with a single-channel pipette are multiplied by 96. Achieving accurate, reliable and reproducible pipetting results is even more challenging. Considerations on the technique are of greatest importance here. One technique to face some problematic liquids such as viscous or volatile liquids is “reverse pipetting technique”. In contrast to classic “forward pipetting technique” the 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. This technique can be done with manual and electronic pipettes. For the 96-channel pipettors ep*Motion* 96 and ep*Motion* 96xl the mode “reverse pipette” has also been created to enable the technique for problematic liquids.
In table 1 we collected handling advices for each liquid type discussed in this white paper. Additionally we recommend the mode that is most beneficial for mastering the various challenges. We give tips to reduce the difficulties arising from problematic liquids. These hints will help to increase pipetting accuracy and precision with problematic liquids with the ep*Motion* 96 and 96xl.

Table 1: Tips for successful transfer of different liquids including problematic liquids using the correct modes and additional considerations

Liquid examples	Liquid type (properties)	Optimal mode	Tips for successful liquid transfer
Aqueous solutions	Water-like properties	All modes	> Follow the ep*Motion* 96 general pipetting practices
Ethanol 70 % 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 to liquid dripping
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 ep*Motion* 96 for highly viscous liquids such as glycerol 99 % is not recommended. A positive displacement pipetting system such as a Multipette®/Repeater® is a better option.
Tween 20 (0,1 % and 1 %)	Detergent	Reverse pipette	> Follow the ep*Motion* 96 general pipetting practices
		Multidispense mode	> No blow-out when re-using the tips > Blow-out would lead to foam formation
		Pipette mode	> 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 ep*Motion* 96 general pipetting practices
		Multidispense	> Do not re-use tips > No blow-out when re-using the tips > Blow-out would lead to bubble formation > Solutions may stick to the outside of the tip > Ensure tip is only immersed to 1-2 mm below the liquid surface > If bubbles are present after dispensing in the plate centrifuge at 200 rcf for 5 minutes without lid for removal
DMSO (1 % -10 %)	Dense and viscous	Reverse pipette	> Follow the ep*Motion* 96 general pipetting practices
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 ep*Motion* 96 for highly viscous liquids such as glycerol 99 % is not recommended. A positive displacement pipetting system such as a Multipette®/Repeater® is a better option.

References

[1] Baier U, Weißbach F. Focus on Fokusheft: Schaumbildung in Biogasanlagen. Leipzig: DBFZ; 2015.

Ordering information

Description	Order no. EU	Order no. North America
ep*Motion*® 96, semi-automated electronic pipette for parallel 96 channel microplate processing, (without iPod® controller), 100 - 240 V ± 10 %/ 50 - 60 Hz ± 5 %, 0.5 - 300 µL	5069 000.012	5069000004
ep*Motion*® 96 with 2-position slider, semi-automated electronic pipette for parallel 96 channel microplate processing, (without iPod® controller), 100 - 240 V ± 10 %/ 50 - 60 Hz ± 5 %, 0.5 - 300 µL	5069 000.110	5069000101
ep*Motion*® 96xl, semi-automated electronic pipette for parallel 96 channel microplate processing, (without iPod® controller), 100 - 240 V ± 10 %/ 50 - 60 Hz ± 5 %, 5 - 1.000 µL	5069 000.217	5069000209
ep*Motion*® 96xl with 2-position slider, semi-automated electronic pipette for parallel 96 channel microplate processing, (without iPod® controller), 100 - 240 V ± 10 %/ 50 - 60 Hz ± 5 %, 5 – 1.000 µL	5069 000.314	5069000306
Upgrade kit 2-position slider	5069 074.008	5069074008

Improve your plate assays and handling of problematic liquids.

About Eppendorf

Eppendorf is a leading life science company that develops and sells instruments, consumables, and services for liquid-, sample-, and cell handling in laboratories worldwide. Its product range includes pipettes and automated pipetting systems, dispensers, centrifuges, mixers, spectrometers, and DNA amplification equipment as well as ultra-low temperature freezers, fermentors, bioreactors, CO_2 incubators, shakers, and cell manipulation systems. Associated consumables like pipette tips, test tubes, microtiter plates, and disposable bioreactors complement the instruments for highest quality workflow solutions.

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