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APPLICATION NOTE No. 192

epT.I.P.S.® LoRetention – Determination of Liquid Residues in Pipette Tips Following Pipetting of Solutions Containing Detergents

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Abstract

For physical reasons, pipette tips made from standard materials will always retain a liquid film during pipetting of liquids containing detergents. The extent of the residual film will vary according to application and handling. This effect may lead to loss of valuable sample material as well as impaired dispensing precision.

The experiments described here demonstrate that the Eppendorf epT.I.P.S. LoRetention retain dramatically less residual liquid after use with various test solutions. Consequently, loss of material is minimized and high pipetting precision is maintained.

Introduction

Precision and accuracy during pipetting are the basis for meaningful and reproducible results. In the field of molecular biology, in particular, evermore sensitive methods have been introduced, thus increasing the need for accurate dispensing of very small volumes. Important factors which have an impact on the accuracy of a dispensing system include design, materials and manufacturing, as well as perfect fit of the instrument with the corresponding consumables. In addition, the use of the appropriate system, in combination with the correct technique for each application, must not be neglected [1, 2, 3]. For example, very viscous solutions, as well as solutions with high vapor pressure, call for direct displacement systems rather than air cushion pipettes. Alternatively, the technique of reverse pipetting may be considered.

A further phenomenon is observed upon pipetting solutions containing detergents. These have lower surface tension compared to water, leading to wetting of polypropylene surfaces. For this reason, following dispensing of liquid with a polypropylene pipette tip, a film remains on the tip's surface, the extent of which may vary according to solution, personnel, dispensing speed and quality of the tip. In addition to accuracy and precision being compromised handling is often made even more difficult by the formation of foam. Within molecular biology applications, detergents are present in many enzyme solutions (e.g. restriction enzymes, polymerases, PCR master mixes) and in solutions required for the preparation of nucleic acids and proteins. Apart from the influence on homogeneity and reproducibility of the results, one must consider the fact that with each pipette tip valuable reagents are being wasted.

Eppendorf epT.I.P.S. LoRetention were developed specifically for the applications outlined above. The tips, made from ultra pure polypropylene, are rendered more hydrophobic via a novel plasma manufacturing procedure. Thus, the so-called "low retention" effect is achieved, causing liquids with low surface tension no longer to form a residual film, thus enabling accurate and precise dispensing (Fig. 1). It is important to note that the tips are neither siliconized, nor do they contain any coating, which could leach and interfere with the sample.
In this Application Note, the liquid remaining inside a variety of pipette tips after one pipetting step has been determined. For this purpose, we have used standard detergent solutions commonly found in molecular biology applications. Eppendorf epT.I.P.S. Standard and LoRetention, as well as "low retention" tips by three other manufacturers, were compared.

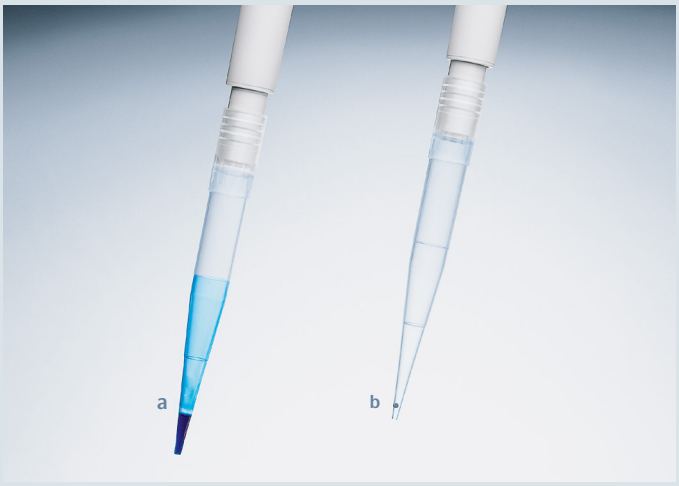

Figure 1: Pipette tips after aspiration and dispensing of a colored detergent-containing solution.
a) Standard tip
b) Eppendorf epT.I.P.S. LoRetention

Material and Methods

Eppendorf epT.I.P.S. Standard and LoRetention 200 µL and 10 µL were used for the experiments described below. In addition "low retention" tips of the 200 µL format by three other manufacturers and of the 10 µL format by two other manufacturers were tested.

The following detergent containing solutions were used: 0.1% Triton® X-100, 1% SDS, MasterMix® (2.5 x) (5-Prime).

Each solution, inside a small container, was placed onto a balance (Mettler Toledo® AG 285, Mettler Toledo SAG 245 P) and the weight was recorded as zero value. Then, 200 µL or 10 µL of liquid were aspirated, respectively, and dispensed back into the container as slowly as possible. Following a pause of approximately 5 seconds, any extra liquid was dispensed via the blow-out. The difference compared to zero value was noted. Each measurement was repeated 10 times. Average and standard deviation were calculated, and the results were converted to percent loss (residual liquid).

Results and Discussion

Figures 2–4 show that wetting of the Eppendorf epT.I.P.S. LoRetention is lowest for both pipette tip sizes (10 µL and 200 µL), as compared to standard tips and "low retention" tips from other manufacturers. In addition, high precision was achieved with all tested solutions. In principle, the percentage of residual liquid is higher in 10 µl tips than in the larger 200 µL tips due to the unfavorable surface-to-volume ratio.
The film of residual liquid remaining inside the pipette tip is most prevalent for the PCR master mix (Fig. 2); the effect is most noticeable due to the fact that in PCR preparation, despite generous calculation, often the PCR master mix prepared is hardly or not at all sufficient. This is due to detergents and other additives typically present in these reagents, such as glycerol, which contribute to higher viscosity. Per each pipetting step, approximately 1.4 mg (12.6%) remain inside a 10 µL pipette tip, and 5.6 mg (2.5%) remain inside a 200 µL tip. The use of epT.I.P.S. LoRetention can strongly reduce these values to 0.6 mg (5.0% inside the 10 µL tip) and 0.7 mg (0.3% inside the 200 µL tip), respectively.

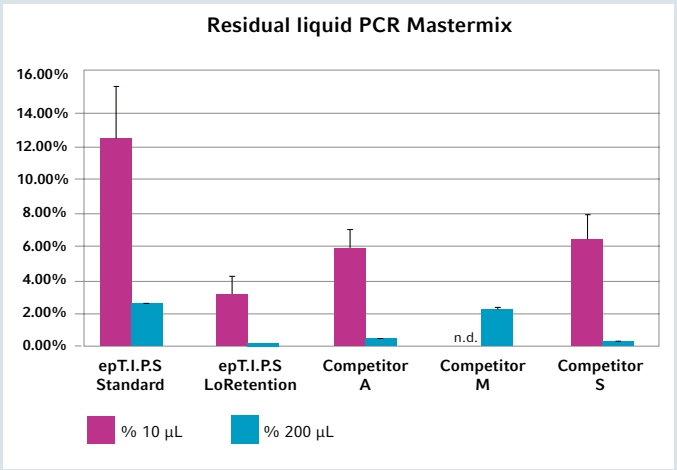

Figure 2: Determination of residual liquid in 10 µL and 200 µL pipette tips for PCR master mix, using the electronic pipette Eppendorf Xplorer®. (n.d.: not determined)

When 1% SDS solution is used, wetting is generally low (Fig. 3). Even under these conditions Eppendorf LoRetention tips achieve superior results, thus demonstrating that high precision dispensing is possible. In contrast, the "low retention" tips made by other manufacturers perform worse than Eppendorf Standard tips.

In addition to the results obtained with Triton X-100 (0.1%) using the electronic pipettes, Fig. 4 also shows results obtained with the manual Eppendorf Research® plus Pipette.

Under all conditions the residual wetness is lowest for the epT.I.P.S. LoRetention while at the same time maintaining high precision. Furthermore, when using epT.I.P.S. LoRetention the influence of different types of pipettes is hardly noticeable. Residual liquid is considerably higher when using the standard tips, as well as "low retention" tips by other manufacturers. Here, a difference could be detected between manual and electronic pipetting, thus demonstrating that this variable, which may express itself in inconsistent pipetting speed or other handling factors, may influence the dispensing result. This effect is minimized when using Eppendorf LoRetention tips.

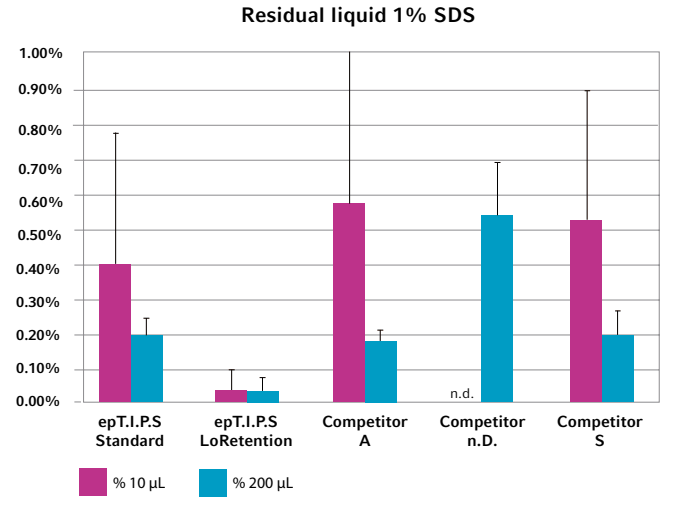

Figure 3: Determination of residual liquid using 10 µL and 200 µL pipette tips in combination with the electronic pipette Eppendorf Xplorer® for a 1% SDS solution. (n.d.: not determined)

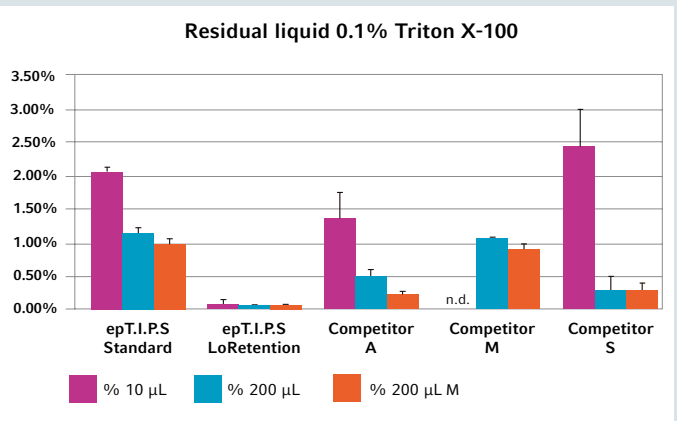

Figure 4: Determination of residual liquid in 10 µL and 200 µL pipette tips for 0.1% Triton X-100 solution, using the electronic pipette Eppendorf Xplorer and the manual Eppendorf Research plus (M). (n.d.: not determined)

Conclusion

Superior results could be demonstrated for the Eppendorf epT.I.P.S. LoRetention regarding dispensing of various solutions containing detergents. Detergents are found in many enzyme solutions, mostly polymerases and PCR master mixes, and they are also used during the isolation of nucleic acids and proteins.

All these applications rely on high accuracy and reproducibility during all pipetting steps. Thus, the use of Eppendorf LoRetention tips will help to minimize the loss of valuable sample materials and expensive reagents, achieving efficient utilization of all materials.

Literature

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