Applications

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Technical Report

Eppendorf epT.I.P.S.® LoRetention – Comparison of low retention pipette tips via simple and fast absorbance measurements in the BioPhotometer™ plus

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Abstract

The wetting properties of detergent solutions are unlike those of water. For this reason, often an almost invisible film remains on the pipette tip during dispensing of such solutions. The use of "low retention" tips may be helpful in minimizing this effect.
By employing a simple test system (absorbance measurements in the Eppendorf BioPhotometer plus), the properties regarding flow behavior of different "low retention" pipette tips were examined. It became apparent that the Eppendorf ep.T.I.P.S.® LoRetention consistently retained the smallest amount of liquid, while achieving the highest pipetting precision, when compared to tips from other manufacturers.

Introduction

In comparison with water, the surface tension of detergent solutions is low. For this reason, such solutions do not drip well off plastics like polypropylene; instead, wetting will occur. Thus, following dispensing, a liquid film remains on the surface of the pipette tip. Since most detergent-containing solutions in the laboratory (e.g. enzyme solutions) are transparent and dye-free, the film remaining inside the tip is hardly visible. Hence, neither the loss of sample, nor the diminished accuracy and precision, are noticed during the pipetting process.

Using colored solutions, normally invisible drops and films, which remain inside the pipette tip, may be made visible. Thus, differences between the various types of pipette tips are now visible to the naked eye during the pipetting process, which aids in making the decision which pipette tip is the most suitable for a given solution. Smaller differences between the pipette tips may be brought to light by absorbance measurements in a photometer. In addition, repeat measurements enable the calculation of pipetting precision.

This Technical Report compares the Eppendorf epT.I.P.S. LoRetention to Standard Eppendorf epT.I.P.S., as well as to "low retention" tips made by other manufacturers via an absorbance test in the BioPhotometer plus. These experiments demonstrate how the properties of pipette tips may be made visible during the use of detergent-containing solutions using a simple procedure.

Materials and Methods

For these experiments, sterile standard Eppendorf Dualfilter T.I.P.S. and Eppendorf Dualfilter T.I.P.S. LoRetention of the 200 µL size were tested, as well as seven "low retention" tips from other manufacturers. The electronic pipette Eppendorf Research pro 20-300 µL was used to pipet the samples. This electronic pipette was chosen to exclude all manual influence on the pipetting process, such as varying pipetting speed, thus ensuring the highest possible reproducibility of the results [1]. The pipette was programmed according to the user manual [2]. The programs and the chosen options are listed in table 1.

First, 100 µL distilled water were pipetted into a UVette using the Standard Eppendorf Dualfilter T.I.P.S (Program 1), thus defining the blank value in the BioPhotometer plus. Subsequently, using a pipette tip to be tested, 100 µL of the colored detergent solution were aspirated and dispensed back into the same tube by pipetting against the inside wall of the tube (Program 2). In order to avoid re-aspiration of the liquid from the tube wall, the following dispensing technique was performed using the Research pro pipette, which is equivalent to the dispensing technique using a manual pipette:

Table 1: Programming of the Eppendorf Research pro

Program 1:
Pipetting of 100 µL water into the UVette
- Aspiration and dispensing speed: Highest
- Option: Standard (comparable to a manual pipette)

Program 2:
Aspiration and dispensing of 100 µL detergent solution
- Aspiration and dispensing speed: Lowest
- Option: Standard (comparable to a manual pipette)

Program 3:
Rinsing of the residual liquid into the water inside the UVette (100 µL)
- Aspiration and dispensing speed: Lowest
- Option: Rinse (RNS) = Threefold rinsing and mixing

A 0.1 % Triton® X-100 solution, dyed with Brilliant Blue FCF, was used as the test solution. The absorption measurements were performed in the Eppendorf BioPhotometer plus using the Eppendorf UVette (UV transparent single-use cuvettes). The method "Absorbance" was chosen at 595 nm [3], a wavelength at which the dye absorbs well in the neutral to basic pH range.

The dispensing button was continually depressed during the dispensing of the detergent solution; only after the tip was removed from the solution, it was released [2] (table 2). Subsequently, the remaining liquid was rinsed from the pipette tip into the water which had previously been placed into the UVette (Program 3).

The UVette was measured in the BioPhotometer plus at a path length of 10 mm. The absorbance value represents the amount of detergent solution remaining inside the pipette tip. 10 values were obtained for each type of tip, from which the averages and standard deviations were calculated.

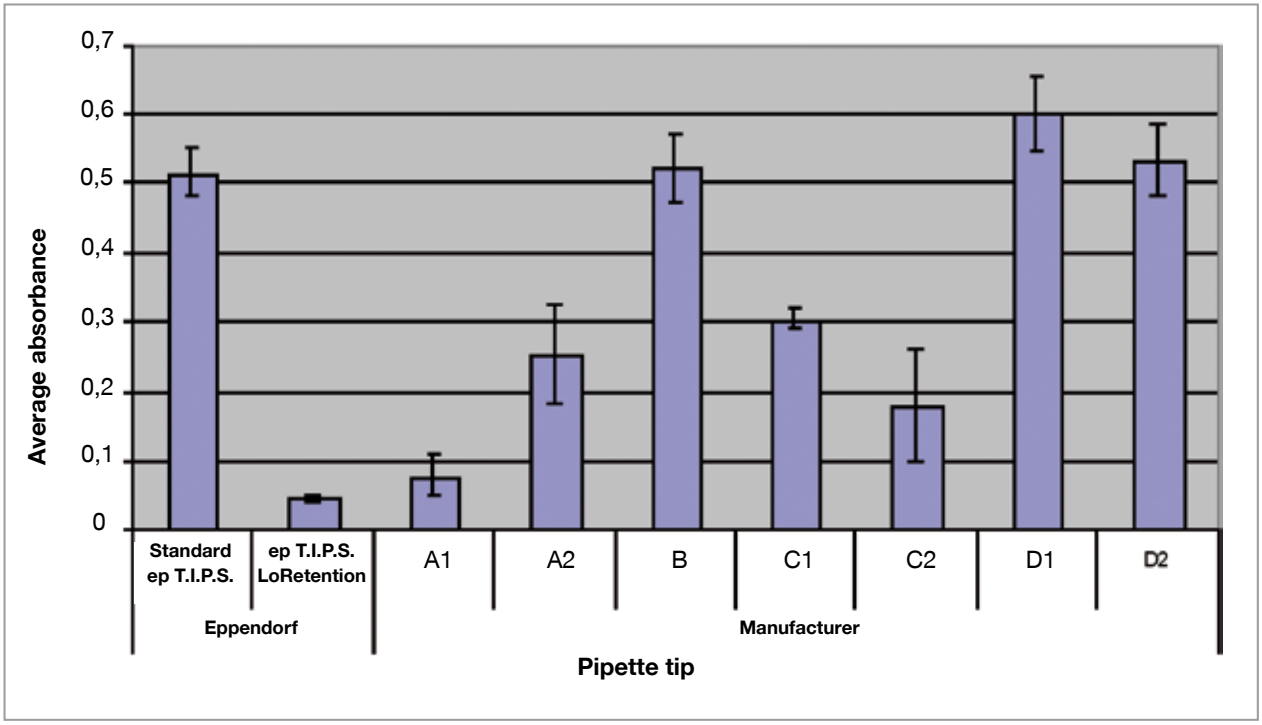
Table 2: Directions for carrying-out the pipetting experiments

Important notes:
- Immerse the tip only a few mm during aspiration.
- Dispense the liquid along the inside wall of the tube.
- In order to avoid re-aspiration of the liquid, release the dispensing button only after the liquid has been dispensed and the pipette tip was removed from the tube.
- Avoid air bubbles in the UVette.

Results and Discussion

Figure 1 shows the results of the absorbance measurements. The higher the absorbance, the more residual liquid remained inside the pipette tip. epT.I.P.S. LoRetention achieved the lowest absorbance values and the smallest standard deviation. These tips retained only small amounts of liquid, while at the same time achieving high reproducibility. All other "low retention" tips tested yielded considerably higher absorbance values, i.e. more residual liquid inside the tip. Tip A's absorbance values are 1.7 times as high, whereas those of tip D1 are 13 times higher than the absorbance values of epT.I.P.S. LoRetention. Tips B, D1 and D2 are at a level similar to that of Eppendorf Standard epT.I.P.S., i.e. tips without "low retention" properties. These tips do not offer an advantage during pipetting of this detergent-containing solution compared to standard tips.

Figure 1: Average absorbance values per tip type
Values were calculated from 10 individual measurements performed in the BioPhotometer plus. The error bars represent the standard deviations.



Tips A2 and C2 produced particularly high standard deviations at relatively low absorbance. In these cases, the amount of residual liquid inside the tip varied considerably between individual measurements, which was already visible to the naked eye. Major variations between tips present a source of error during the pipetting process.

These experiments were also performed using a manual pipette. A very controlled and slow dispensing speed will ensure further reduction of sample residue inside the tip; however, the variation between values will increase, due to additional potential human error sources. Even throughout this experiment, epT.I.P.S. LoRetention continued to show the lowest retention values (data not shown).

The results demonstrate that absorbance measurements performed in the BioPhotometer plus are suitable to distinguish differences in the flow properties of pipette tips. In many cases, these differences were already visible in the tip by the naked eye. By performing ten individual measurements, the homogeneity of a particular type of tip (for example, from one tip box) may also be determined using this fast and simple test system.
Please note, however, that this experimental design is not suitable for calibration of dispensing systems in accordance with EN ISO 8655 [4].

Conclusions

Within this report, a test was demonstrated which provides a fast and simple measurement of the extent to which colored residual liquids are retained inside a given pipette tip. This test enables the user to determine whether a certain pipette tip is suitable for the solution on hand. The experiments performed here verify, as previously shown elsewhere [5], that the Eppendorf epT.I.P.S. LoRetention have the lowest wetting values, thus providing superior flow behavior compared to “low retention” tips made by other manufacturers. Due to minimized sample loss and high tip homogeneity when using Eppendorf epT.I.P.S. LoRetention for pipetting detergent-containing solutions, sophisticated experiments may be performed in a reproducible and reliable fashion.

Literature

[1] Application Note 92: Comparison of pipetting behavior of the electronic pipette Eppendorf Research pro and the manual pipette Eppendorf Research via an enzyme linked immunosorbent assay (ELISA) (www.eppendorf.com)
[2] User manual Research pro (www.eppendorf.com)
[3] User manual BioPhotometer plus (www.eppendorf.com)
[4] Eppendorf SOP - Standard Operating Procedure for Pipettes (www.eppendorf.com)
[5] Application Note 192: Eppendorf epT.I.P.S. LoRetention – Determination of residual liquid amounts in pipette tips following pipetting of solutions containing detergents (www.eppendorf.com)

Ordering information

Description	Order no. international	Order no. North America
ep Dualfilter T.I.P.S. LoRetention, PCR clean, sterile and pyrogen-free (10 Racks à 96 Tips = 960 Tips)		
0,1 – 10 µL S	0030 077.610	022493000
0,5 – 20 µL L	0030 077.628	022493002
2 - 100 µL	0030 077.644	022493006
20 – 300 µL	0030 077.636	022493004
50 - 1000 µL	0030 077.652	022493008
epT.I.P.S. LoRetention Reloads, PCR clean (10 Trays à 96 Tips = 960 Tips)		
0,1 – 10 µL S	0030 072.006	022493010
0,5 – 20 µL L	0030 072.014	022493012
2 - 200 µL	0030 072.022	022493014
50 - 1000 µL	0030 072.030	022493016
epT.I.P.S. LoRetention Reloads, Eppendorf Qualität (10 Trays à 96 Tips = 960 Tips)		
0,1 – 10 µL S	0030 072.049	022493018
0,5 – 20 µL L	0030 072.057	022493020
2 - 200 µL	0030 072.065	022493022
50 - 1000 µL	0030 072.073	022493024
epT.I.P.S. LoRetention Racks, PCR clean (10 Racks à 96 Tips = 960 Tips)		
0,1 – 10 µL S	-	022493026
0,5 – 20 µL L	-	022493028
2 - 200 µL	-	022493030
50 - 1000 µL	-	022494032
BioPhotometer plus	6132 000.008	952000006
UVette, individually packaged single cuvettes, certified RNase-, DNA- and protein free, 80 pcs.	0030 106.300	952010051
UVette routine pack, Eppendorf Quality purity level, reclosable box of 200 pcs.	0030 106.318	952010069
Eppendorf Research pro, 20-300 µL, single-channel pipette with charging adapter	4860 000.038	022461320

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