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ep Dualfilter T.I.P.S.® SealMax – Determination of sample recovery rate of self-sealing filter tips in case of over-pipetting

Lu Foong Teng[1], Detlef Hempfling[2]
[1]Eppendorf Asia Pacific Headquarters, Kuala Lumpur, Malaysia
[2]Eppendorf Instrumente GmbH, Hamburg, Germany

Abstract

In case of accidental over-pipetting, self-sealing filter tips provide additional protection against contamination by forming a sealed barrier upon contact with the sample liquid, preventing it from passing through the filter. The ability to recover the sample as much as possible thereafter is questionable. An investigation was carried out to determine the sample recovery rate of self-sealing filter tips following over-pipetting. Results show that nearly complete sample recovery is achievable using ep Dualfilter T.I.P.S. SealMax. It has the highest recovery rate and reproducibility irrespective of the filter tip size when compared with two other manufacturers of self-sealing filter tips.

Introduction

During pipetting using an air-cushion pipette, there is potential risk of aerosols formation within the pipette system. This presents a concern since it leads to contamination of the pipette and cross-contamination between samples. Filter tips are commonly used as a solution to address this issue.

Another source of contamination, unforeseen and often neglected, is the ingress of the sample liquid into the pipette due to improper handling or accidental over-pipetting resulted from incorrect tip size used or wrongly set volume. The occurrence of such risk calls for the need of a filter tip that additionally provides protection against contamination by the sample liquid, apart from guarding against aerosols contamination. This can be realized by having a filter which seals upon contact with liquid, forming a barrier that prevents the liquid from penetrating through the filter and contaminating the pipette (Figure 1). Filter tips behaving in this way are generally known as “self-sealing” filter tips.

(a) ep Dualfilter T.I.P.S. (b) ep Dualfilter T.I.P.S. SealMax

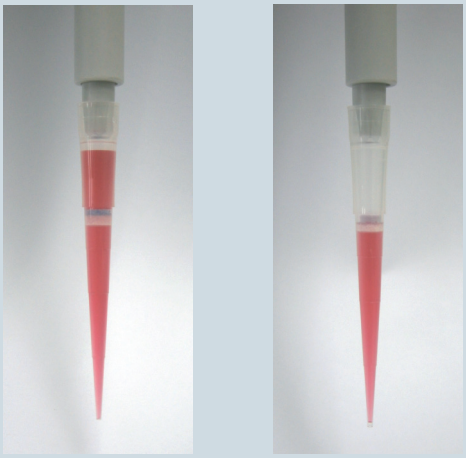

Figure 1: Both (a) and (b) show pipetting of 300 µL sample using a 100 µL filter tip size.
(a) A standard non self-sealing filter tip does not effectively prevent liquid from penetrating through the filter in case of excessive over-pipetting. The example shown here is the standard ep Dualfilter T.I.P.S.
(b) ep Dualfilter T.I.P.S. SealMax forms a reliable barrier against liquid should accidental over-pipetting occur.

With regard to the preceding purpose, the filter has to seal effectively against the incoming liquid. On the other hand, the self-sealing filter should then allow release of the sample for recovery. The ability to enable recovery of sample as much as possible presents a tough challenge. Sample loss is especially critical in situations where there is limited availability of sample amount, preparation of sample is difficult and time-consuming or when working with expensive reagents.

The investigation described herein aims to determine the amount of sample liquid that can be recovered from ep Dualfilter T.I.P.S. SealMax following sealing of the liquid by the filter in case of over-pipetting. The sample recovery rate is also compared with that of two other manufacturers of self-sealing filter tips.

Materials and Methods

All sizes of ep Dualfilter T.I.P.S. SealMax were tested for sample recovery rate following over-pipetting. 10 µL and 200 µL self-sealing filter tips from manufacturer G and A were tested alongside for comparison.

The pipette–tip combination stated in Table 1 was used to simulate the situation of over-pipetting. Electronic pipette (Eppendorf Xplorer®) was used to exclude all manual influences on the pipetting process. Exceptions are for testing 10 µL and 20 µL filter tip size where Eppendorf does not offer an electronic pipette with nominal volume 20 µL. Hence, a manual pipette (Eppendorf Research® plus) was used instead, as indicated in Table 1.

Table 1: Overview of pipette-tip combination tested to simulate the situation of over-pipetting

Filter tip size	Pipette at nominal volume	Pipette type
10 µL	20 µL with reverse pipetting	Manual
20 µL	20 µL with reverse pipetting	Manual
100 µL	300 µL with forward pipetting	Electronic
200 µL	300 µL with reverse pipetting	Electronic
300 µL	300 µL with reverse pipetting	Electronic
1 000 µL	1 200 µL with reverse pipetting	Electronic

Distilled water was used as test liquid. All test conditions were in accordance with Eppendorf SOP [1] while the test procedure was modified to accommodate the aim of this investigation.

The weighing vessel with added test liquid was placed on an analytical balance and tared to zero. Referring to Table 1, test liquid was aspirated into the pipette tip upon which over-pipetting occurred. The reading on the balance was recorded as Value 1, which corresponds to the actual amount of liquid aspirated. Then, the balance was tared to zero again. Test liquid was recovered by dispensing back into the weighing vessel using the pipette. The reading on the balance was recorded as Value 2, which corresponds to the amount of liquid recovered. The waiting time period between aspiration and dispensing was kept at 10 seconds.

Sample recovery rate was determined as follows:

$$\text{Percentage sample recovery (\%)} = \frac{\text{Value 2}}{-\,(\text{Value 1})} \times 100$$

For each manufacturer and each test filter tip size, sample recovery rate measurement was repeated ten times using a new tip for each measurement. The average percentage of sample recovery and standard deviation were calculated.

Results and Discussion

The sample recovery rate of all tip sizes of the ep Dualfilter T.I.P.S. SealMax following over-pipetting was determined (Figure 2). It is clearly shown that ep Dualfilter T.I.P.S. SealMax enables nearly all of the sample volume (> 95%) to be recovered even when the sample liquid comes into contact with the filter, which causes the filter to seal and prevent the liquid from passing through. Maximum sample recovery can be achieved by simply dispensing the sample out of the tip using the pipette. No cumbersome additional work steps, for example cutting the tip, are required. The standard deviation between individual tips of the same size, as represented by the error bar, was also very small. This indicates the high reproducibility of sample recovery rate from tip to tip. Both the recovery rate and its reproducibility were consistent across all the different tip sizes of ep Dualfilter T.I.P.S. SealMax.

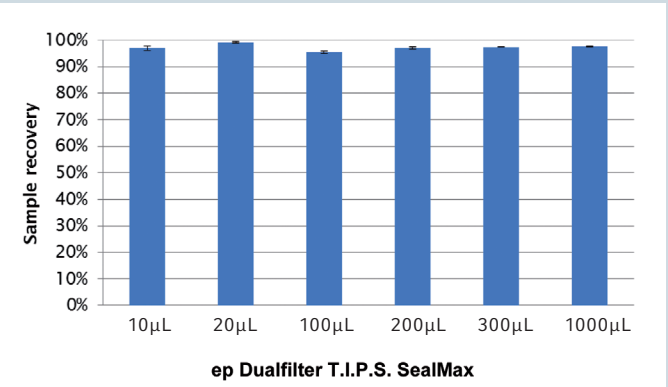

Figure 2: Sample recovery rate of ep Dualfilter T.I.P.S. SealMax following over-pipetting. Values presented are average percentage of sample recovery calculated from 10 individual measurements. The error bar represents standard deviation.

In order to assess the sample recovery rate performance of ep Dualfilter T.I.P.S. SealMax, 10 µL and 200 µL tip sizes were compared with that of two other manufacturers of self-sealing filter tips (Figure 3).

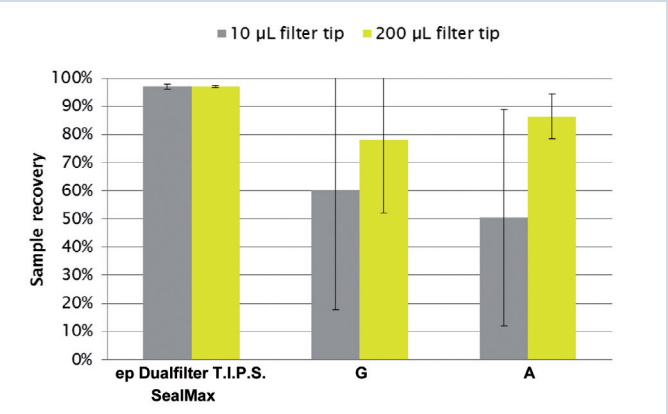

Figure 3: Comparison of sample recovery rate between different manufacturers of self-sealing filter tips. Values presented are average percentage of sample recovery calculated from 10 individual measurements. The error bar represents standard deviation.

Filter tips from manufacturer G and A demonstrated lower sample recovery rate than ep Dualfilter T.I.P.S. SealMax. This was particularly pronounced for the small tip size 10 µL with a difference of 37% lower for manufacturer G and 47% lower for manufacturer A. For 200 µL filter tip, the difference was smaller, which was 19% and 11% lower for manufacturer G and A respectively. Looking at each manufacturer G and A individually, there was considerable difference in the recovery rate between 10 µL and 200 µL filter tips. Such finding suggests dependency of the sample recovery rate on the filter tip size. However, for ep Dualfilter T.I.P.S. SealMax, the recovery rate remained high and consistent regardless of the filter tip size.

Exceptionally large standard deviations were found for both 10 µL and 200 µL filter tips from manufacturer G and 10 µL filter tip from manufacturer A. The sample recovery rate varied substantially from one tip to the other, thus implicating lower reliability of these tips in this aspect.

Presumably, three possible factors contributing to lower recovery rate and reproducibility may be derived based on the results obtained and observation made during this investigation. (i) Sample can be lost due to absorption by the filter material. A hydrophobic filter will greatly minimize the influence of this factor, as for ep Dualfilter T.I.P.S. SealMax. (ii) Should the self-sealing mechanism of the filter also block the air flow within the pipette system, the sample cannot be recovered by simple dispensing or only partial recovery is possible. Filter tips from manufacturer G and A appear to represent this. (iii) The possibility that the sample properties or behavior is altered after coming into contact with the self-sealing filter cannot be ruled out. This phenomenon was observed for filter tips from one of the two other manufacturers tested. The sample liquid became slightly viscous and had tendency of foam formation. During recovery, some liquid was retained as droplets or bubbles on the internal tip wall (Figure 4).

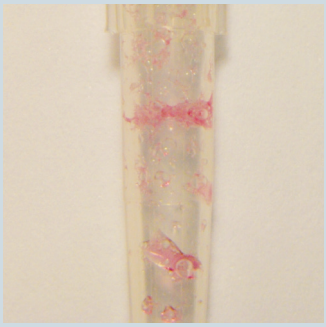

Figure 4: Sample liquid became slightly viscous and had tendency of foam formation after coming into contact with the filter (filter tip from one of the two other manufacturers tested).
This observation was made during the investigation, in which distilled water (colorless) was used as test liquid. For visualization, the picture shows test using distilled water with added red dye.

High and reproducible sample recovery rate following over-pipetting becomes exceptionally critical in processes dealing with valuable samples. Limited sample amount is a typical situation, among others, in forensics and diagnostics. Many a time, there is only one chance for investigation and therefore, analysis must be done right the first time and each time. Using a filter tip that reliably guards against contamination while it simultaneously ensures maximum and easy recovery, despite unpredictable pipetting mistake, will secure the sample for continued analysis. Furthermore, minimum downtime or unnecessary repetition is avoided. The diagnosis of diseases, for example, demands fast analysis result so that decision for implementing a suitable treatment can be made in time. Reliable sample recovery also plays a significant role when it comes to handling samples for which preparation or test procedures are cumbersome and lengthy. Some exemplary fields include viral studies, drug discovery and development, metagenomics, immunological assays as well as archeological examination.

Conclusion

In addition to providing dual protection against aerosols and biomolecules, ep Dualfilter T.I.P.S. SealMax builds a reliable protection against liquid. Of equal importance is that it enables almost all of the samples to be recovered even after coming into contact with the filter. In the context of this investigation, high reliability of the filter tip with respect to sample recovery rate and its reproducibility in case of over-pipetting is proven.

Although other self-sealing filter tips may seemingly exhibit similar performance of forming an effective barrier against liquid, it is clearly shown here that there are large differences in the sample recovery rate and reproducibility. The use of ep Dualfilter T.I.P.S. SealMax will constantly ensure saving of valuable samples, costs and time.

References

[1] Eppendorf SOP – Standard Operating Procedure for Pipettes. www.eppendorf.com/SOP

Ordering information

Product	Color code	Order no.
ep Dualfilter T.I.P.S.® SealMax, Racks, PCR clean / 10 x 96 tips		
0.1 – 10 µL S, 34 mm	dark gray	0030 077.806
0.5 – 20 µL L, 46mm	light gray	0030 077.814
2 – 100 µL, 53mm	yellow	0030 077.822
2 – 200 µL, 55mm	yellow	0030 077.830
20 – 300 µL, 55mm	orange	0030 077.849
50 – 1,000 µL, 76mm	blue	0030 077.857

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