APPLICATION NOTE No. 398

Save Samples and Reagents with the Repeater®/Combitips® System

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Abstract

Total filling volumes of Combitips advanced dispenser tips were measured. It was shown that minimal liquid volumes were needed additionally for reverse stroke and remaining stroke.

In addition, positive displacement tips from other manufacturers were tested alongside the Combitips advanced, and the ratios between filling volume and maximum volume were analyzed.
It was shown that Combitips advanced had the least excess volume and therefore the highest usable volume of all tested tips. The Repeater and Combitips advanced system saves samples and reagents by optimal correlation of technique, tip design, and shape.

Introduction

The mechanical multi-dispenser Repeater M4 and the electronic versions Repeater E3/E3x, in combination with Combitips advanced, form a complete system for dispensing applications in the laboratory.
Generally, the Repeater/Combitips positive displacement system is operated by aspiration of the liquid followed by dispensing in multiple steps. To ensure maximum accuracy for the first and final dispensing step, the system aspirates excess liquid to perform a reverse as well as a remaining stroke.
After liquid aspiration, pressing the dispenser lever (Repeater M4) or actuate key (Repeater E3/E3x) triggers the reverse stroke which is discarded. During this step, the system enters a defined starting position to dispense each step equally. During the final dispensing step, the piston does not reach its lowest position. The residual liquid remaining in the tip can be recovered by the subsequent remaining stroke.
Since reverse and remaining stroke are not considered dispensing steps, more liquid needs to be aspirated into the tip. This fact needs to be considered when working with liquids which are available in limited quantities and technical specifications must take this effect into account.
In this application note, the excess volume, which is required to fill the tip entirely in addition to the dispensable volume, is determined using water. The data obtained regarding the filling volumes form the basis for calculating the total amount of solution required for any given application. Furthermore, the ratio between the filling volume and the volume dispensed by the Repeater M4 and Combitips advanced system will be presented in comparison to mechanical systems by two other manufacturers.

For standardization, distinct terms are used in this document. Below, the terms used for each step during a dispensing application and the description of filling volumes are explained. Figure 1 shows an illustration of the different dispensing steps.

Filling volume Sum of the volumes of reverse stroke, dispensing volume and remaining stroke

Reverse stroke Following liquid aspiration, the piston is brought to a defined start position, dispensing liquid in the process. The reverse stroke is not a dispensing step.

Dispensing volume Volume per dispensing step

Total dispensing volume Sum of all individual dispensing volumes

Remaining stroke After all dispensing steps have been completed, a small amount of liquid remains as safety buffer and is dispensed as remaining stroke.

Maximum dispensable volume The maximum usable volume for all dispensing steps

Nominal volume Maximum dispensing volume indicated by tip size and volume scale of the tip, e.g. 5 mL

Excess volume Volume aspirated by the system in addition to the total dispensing volume. This volume does not contribute to the dispensing volumes but rather ensures volume accuracy at the first and final dispensing step. It includes volume for reverse stroke and remaining stroke.

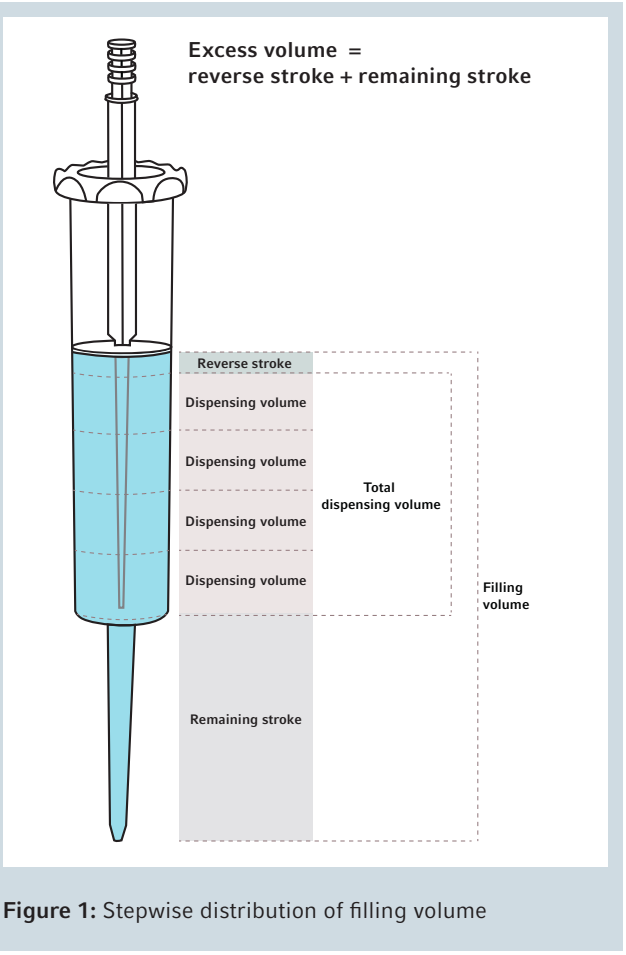

Figure 1: Stepwise distribution of filling volume

Material and Methods

The required filling volume of Combitips advanced dispenser tips was tested with mechanical Repeater M4 and electronic Repeater E3x multi-dispensers. For Repeater E3x, dispensing mode (DIS) was selected and the speed was set to level 5. All sizes of Combitips advanced in the volume range between 0.1 mL and 50 mL were tested with both dispensers.

Comparison of total dispensing volume with other manufacturers
To determine the excess volume required by the Combitips advanced and by tips from two other suppliers, manufacturer R and B, all tips were used in combination with the corresponding manufacturer's mechanical multi-dispenser. The determination was carried out using 2.5 mL and 5 mL tips with 1/10 (10%) and 1/50 (2%) volume as dispensing steps. The tips were filled with distilled water and each dispensing step (reverse stroke, total dispensing volume and remaining stroke) was gravimetrically determined (Analytical balance Cubis® MSE224S-100-DA, Sartorius, Germany). The excess volume was determined based on the difference between the filling volume and maximum dispensable volume.

Results and Discussion

Combitips advanced need minimal excess volume
As shown in Figure 2, the average excess volume while dispensing in Dial position 1 (1/50 nominal volume) ranged between 5-7% of the filling volume for each Combitips advanced dispenser tip.
The excess volume using the Repeater E3x and Repeater M4 are comparable. Only when using 5 mL Combitips advanced the excess volume using Repeater E3x is lower than with the manual dispenser. This effect can be attributed to the ideal piston movement control of the electronic dispenser Repeater E3x.

These data reveal a relatively low variation of the values, as well as highlighting the fact that the Combitips advanced and Repeater M4 or E3x are well-coordinated systems.

Table 1 lists the volumes required to fill any Combitips advanced dispenser tip entirely. As a rule of thumb, it is recommended to provide an additional 10% of the maximum volume of the respective tip. This rule also applies when the tip is not filled completely, because less dispensing steps are needed for an application. The reverse and remaining stroke stay the same as if the tip was filled completely. This information shall be helpful when estimating whether a planned dispensing procedure can be carried out with a given amount of liquid.

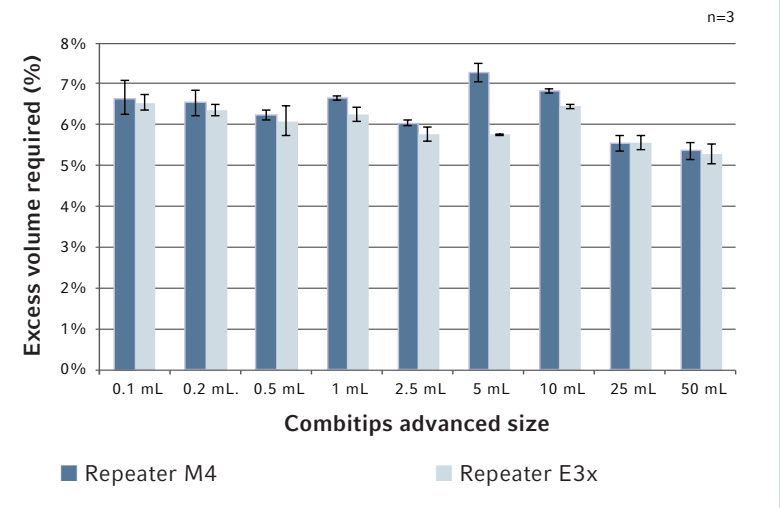

Figure 2: Excess volume required for complete filling of Combitips advanced dispenser tips using Repeater M4 or Repeater E3x ranges between 5.3 and 6.7%.

Table 1: Volume required for complete filling of Combitips advanced using either the Repeater M4 or Repeater E3x.

	Excess volume		Volume required for complete filling	
Combitips advanced size	**Repeater M4**	**Repeater E3x**	**Repeater M4**	**Repeater E3x**
0.1 mL	7 µL	7 µL	107 µL	107 µL
0.2 mL	13 µL	13 µL	213 µL	213 µL
0.5 mL	31 µL	30 µL	531 µL	530 µL
1 mL	66 µL	63 µL	1.1 mL	1.1 mL
2.5 mL	151 µL	144 µL	2.7 mL	2.6 mL
5 mL	364 µL	288 µL	5.4 mL	5.3 mL
10 mL	682 µL	645 µL	10.7 mL	10.6 mL
25 mL	1.4 mL	1.4 mL	26.4 mL	26.4 mL
50 mL	2.7 mL	2.6 mL	52.7 mL	52.6 mL

As shown in Figure 3, manufacturer B's 2.5 mL tips, when dispensing 50 µL, have a comparable excess volume to Combitips advanced. Manufacturer R's 2.5 mL tips meanwhile have a slightly higher excess volume than Combitips advanced.

In contrast, when set to 250 µL dispensing volume, (manufacturer B's largest possible dispensing volume) the excess volume is significantly larger. Also, for manufacturer R the required excess volume is significantly larger than of Combitips advanced with the Repeater M4 multi-dispenser.

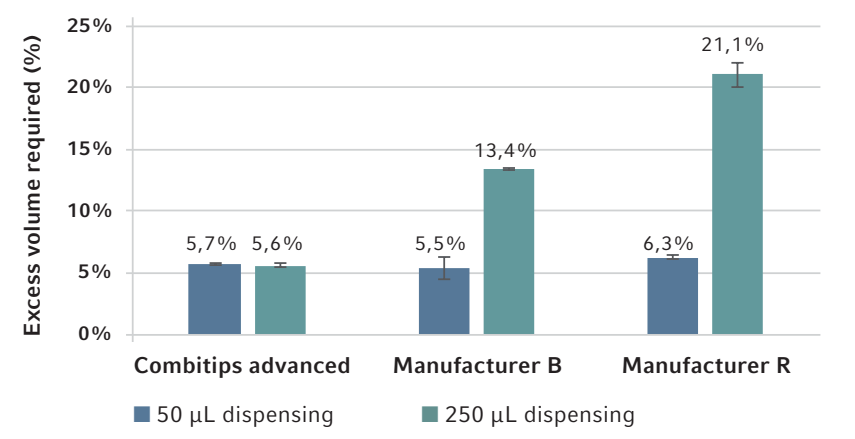

Figure 3: Excess volume for dispensing the nominal volume of 2.5 mL in Combitips advanced and two other manufacturer's dispenser tips. n=3

Table 2 shows the average values determined for 2.5 mL and 5 mL dispensing tips. The table clearly illustrates the discrepancy between the true total dispensing volumes and the nominal volume declared by the other manufacturers in combination with their own instruments. Altogether, a significantly smaller volume can be dispensed with the systems of manufacturer R and B than one would expect based on the respective size specification.

Table 2: Filling and total dispensing volume of Combitips advanced compared to other manufacturer's dispensing tips.

	2.5 mL nominal volume		
	Combitips advanced	**Manufacturer B**	**Manufacturer R**
Filling volume (µL)	2638	2578	2544
Total dispensing volume (µL)	2490	2232	2008
Deviation from declared nominal volume	0.4%	10.7%	19.7%
	5 mL nominal volume		
Filling volume (µL)	5322	5191	5132
Total dispensing volume (µL)	4980	4474	4011
Deviation from declared nominal volume	0.4%	10.5%	19.8%

In contrast, the total dispensing volumes of Combitips advanced and Repeater M4 are in accordance with the nominal volume. In fact, Combitips advanced allow 10 dispensing steps and with this enable usage of the declared nominal volume while competitor's tips fall short of the nominal volume by up to 20%. Manufacturer B technically offers maximum 9 steps leading to a maximum dispensable volume of 2,250 µL and 4,500 µL, respectively. Our data showed that 9 steps could not even be accomplished as only 2,232 µL were available for dispensing (table 2). Manufacturer R only offers 8 dispensing steps meaning a maximum dispensing volume of 2,000 µL or 4,000 µL, respectively. This was shown in our test as the total dispensing volume was 2,008 µL and 4,011 µL.
So, from the technical construction of competitor systems it is impossible to use the complete tip volume, leading to a constant underuse of the tips' potential volume. Multiple fillings are needed to dispense the same amount of liquid while Combitips advanced only need one filling.

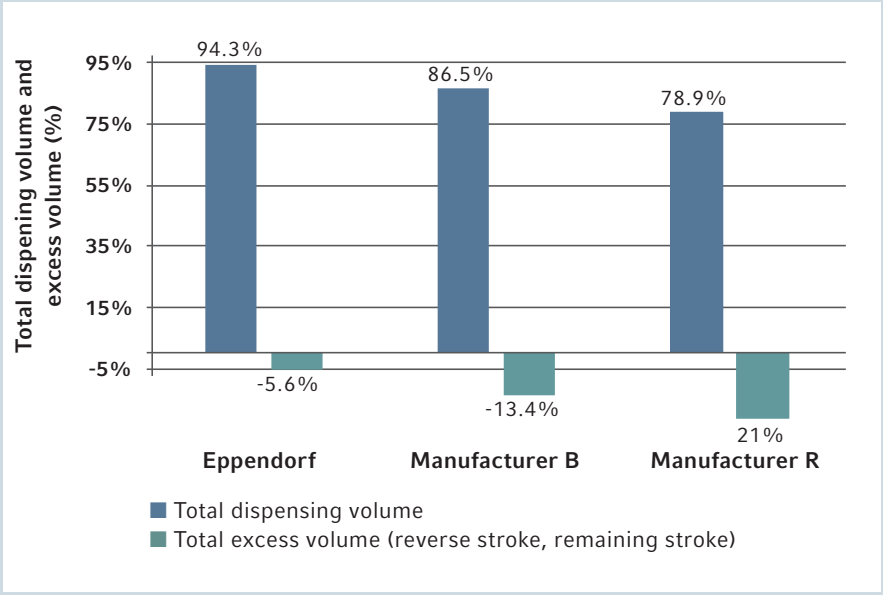

Figure 4: Dispensing volumes in 2.5 mL tips showing that manufacturer B and R have less total dispensing volume and more excess volume than Combitips advanced.

Figure 4 illustrates the percentage distribution of the filling volumes for 2.5 mL tips using 250 µL dispensing steps. For 5 mL tips, the same relationship was determined. A significant amount of liquid needed as reverse and remaining stroke, volume in both competitor tips was visible. If the liquid was not re-dispensed into the source vessel it was wasted. This increased excess volume of sample liquid, reagent solution and raised costs as well as effort.
In contrast using Combitips advanced combined with Repeater M4 enabled usage of the full liquid filling while having only 5% total excess volume.

Conclusion

It was shown that Combitips advanced dispenser tips only need 5-7% additional filling volume. The tested dispenser tips from other manufacturers needed up to 20% additional liquid for complete filling. Furthermore, less than the nominal volume was available for dispensing applications due to setting options and dispenser construction from alternative manufacturers.

Concluding, Combitips advanced have a lower reverse and remaining stroke than the tips of other manufacturers tested. The nominal volume stated on the tip is completely available for dispensing steps. This leads to the maximum sample and reagent usability when working with the Repeater/Combitips system.

Ordering information and technical specifications for Combitips advanced dispenser tips are available at **www.eppendorf.com/combitips.**

For more information and article numbers regarding Eppendorf multi-dispensers, visit **www.eppendorf.com/m4** (mechanical variant) and **www.eppendorf.com/e3** (electronic variants).

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