

# Save Samples and Reagents with Combitips advanced<sup>®</sup> and Multipette<sup>®</sup>/Repeater<sup>®</sup>

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## Abstract

Total filling volumes of Combitips advanced were measured. It was shown that minimum volume was needed additionally for reverse stroke and remaining stroke.

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



## Introduction

The manual hand dispenser Multipette/Repeater M4 and the electronic versions Multipette/Repeater E3/E3x, in combination with Combitips advanced, form a complete system for dispensing applications in the laboratory.

Generally, the 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.

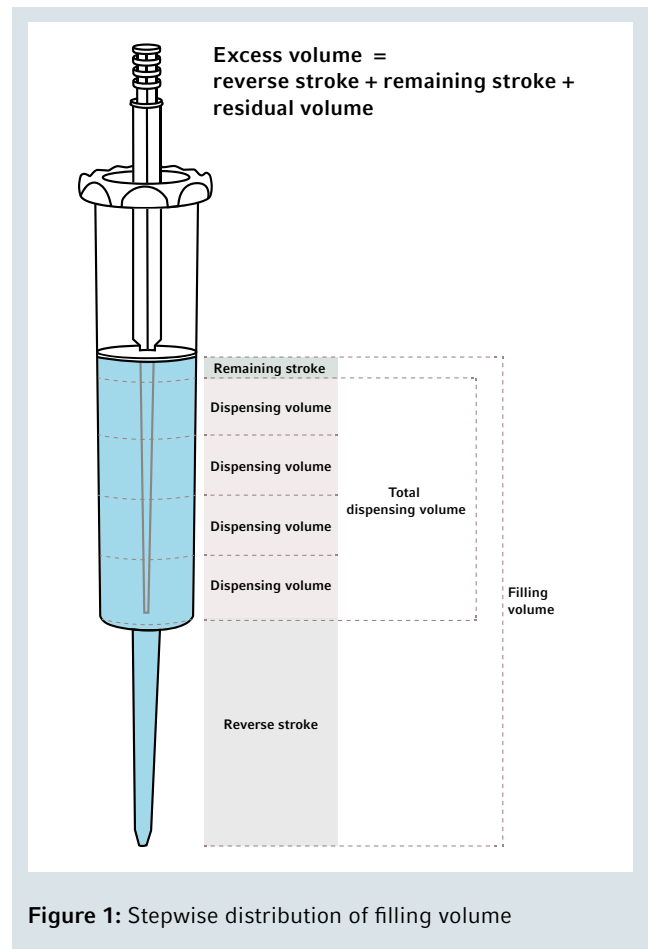
After liquid aspiration, pressing the dispenser lever (manual dispenser) or actuate key (electronic dispenser) triggers the reverse stroke which is discarded. During this step the system enters a defined starting position to dispense each step equally. During the final dispensing step, the piston does 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 Multipette/Repeater M4 and Combitips advanced system will be presented in comparison to manual systems by two alternative 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.

<b>Filling volume</b>	Sum of the volumes of reverse stroke, dispensing volume, remaining stroke and residual volume
<b>Reverse stroke</b>	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.
<b>Dispensing volume</b>	Volume per dispensing step
<b>Total dispensing volume</b>	Sum of all individual dispensing volumes
<b>Remaining stroke</b>	After all dispensing steps have been completed, a small amount of liquid remains as safety buffer and is dispensed as remaining stroke.
<b>Maximum dispensable volume</b>	The maximum usable volume for all dispensing steps
<b>Nominal volume</b>	Maximum dispensing volume indicated by tip size and volume scale of the tip, e.g. 5 mL
<b>Excess volume</b>	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, remaining stroke and residual volume.



## Material and Methods

### Combitips advanced need minimal excess volume

The required filling volume of Combitips advanced was tested with manual Multipette/Repeater M4 and electronic Multipette/Repeater E3x. For Multipette/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 alternative manufacturers

To determine the excess volume required by the Combitips advanced and by tips from two alternative manufacturers, competitor R and B. All tips were used in combination with the corresponding manufacturer's manual 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 residual volume was determined based on the difference between the filling volume and maximum dispensable volume.

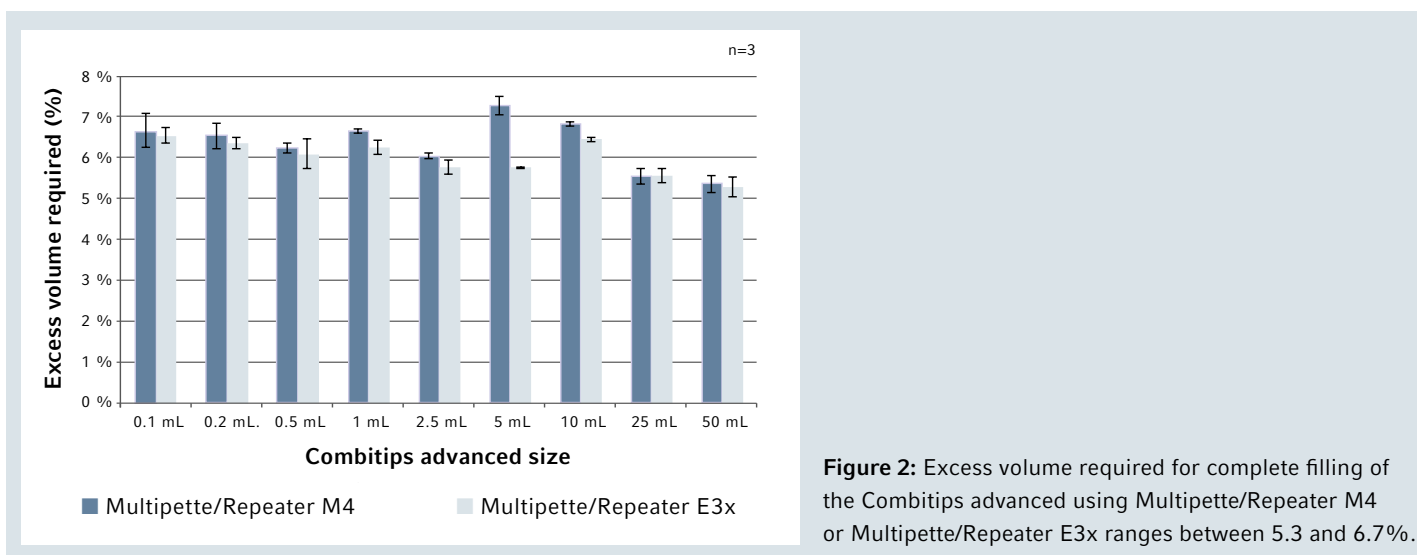
## Results and Discussion

### Combitips advanced need minimal excess volume

As shown in Fig. 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. The excess volume using the Multipette/Repeater E3x and Multipette/Repeater M4 are comparable. Only when using 5 mL Combitips advanced the excess volume using Multipette/Repeater E3x is lower than with the manual dispenser. This effect can be attributed to the ideal piston movement control of the electronic dispenser Multipette/Repeater E3x.

Table 1 lists the volumes required to fill any Combitips advanced 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.

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



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

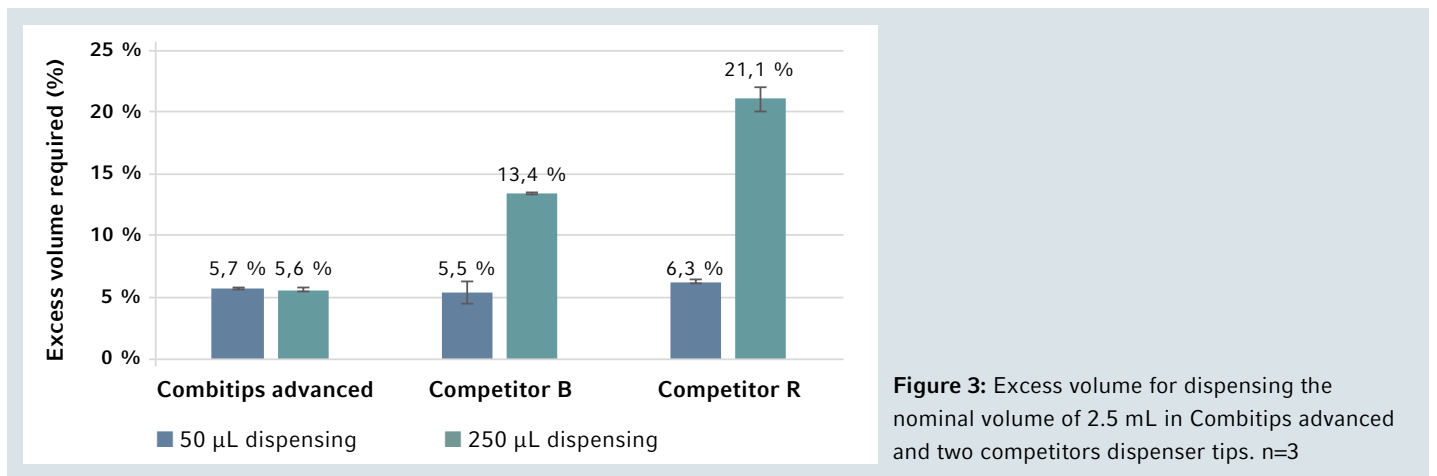
**Table 1:** Volume required for complete filling of Combitips advanced using either the Multipette/Repeater M4 or Multipette/Repeater E3x.

Combitips advanced size	Excess volume		Volume required for complete filling	
	Multipette/Repeater M4	Multipette/Repeater E3x	Multipette/Repeater M4	Multipette/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

**Comparison of total dispensing volume with alternative manufacturers**

As shown in Fig. 3, competitor B’s 2.5 mL tips, when dispensing 50 µL have a comparable excess volume to Combitips advanced. Competitor 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, competitor B’s largest possible dispensing volume, the excess volume is significantly larger. Also, for competitor R the required excess volume is significantly larger than of Combitips advanced and Multipette/Repeater M4 system.



**Figure 3:** Excess volume for dispensing the nominal volume of 2.5 mL in Combitips advanced and two competitors 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 competitors in combination

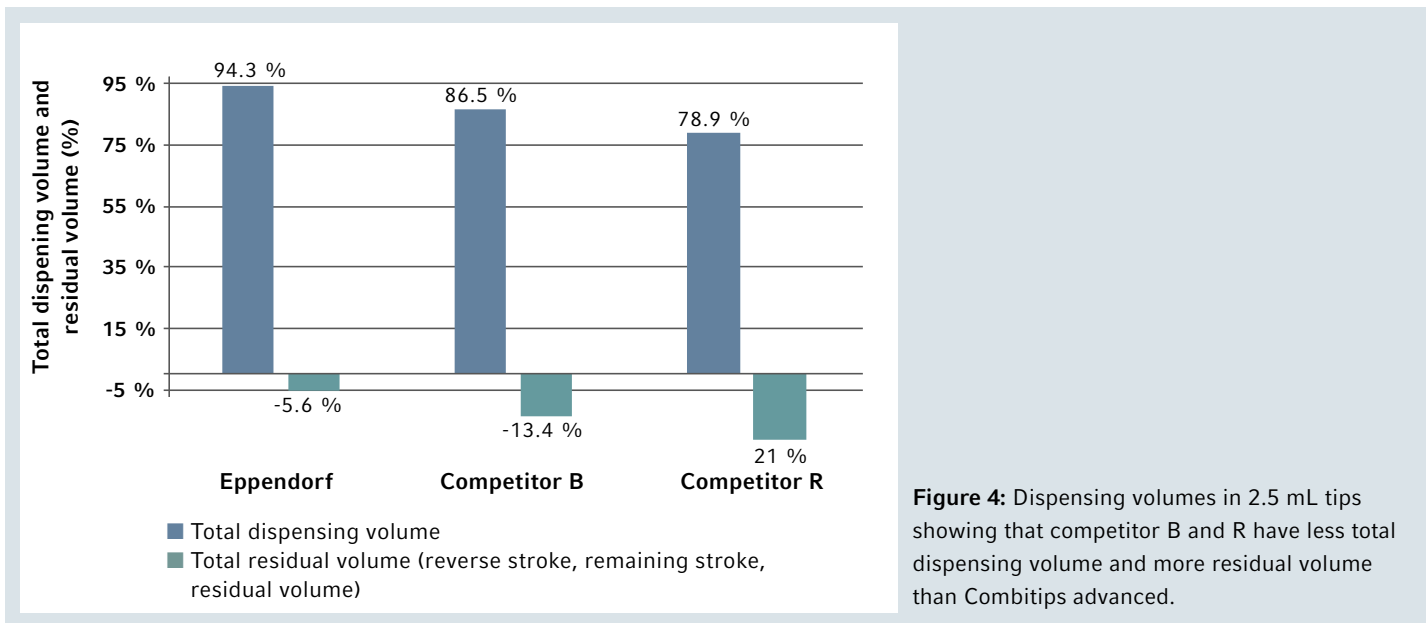
with their own instruments. Altogether, a significantly smaller volume can be dispensed with the systems of competitor R and B than one would expect based on the respective size specification.

**Table 2:** Filling and total dispensing volume of Combitips advanced compared to competitor dispensing tips.

	2.5 mL nominal volume		
	Combitips advanced	Competitor B	Competitor 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		
	Combitips advanced	Competitor B	Competitor R
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 Multipette/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%. Competitor B technically offers maximum 9 steps leading to a maximum dispensable volume of 2,250  $\mu\text{L}$  and 4,500  $\mu\text{L}$ , respectively. Our data showed that 9 steps could not even be accomplished as only 2,232  $\mu\text{L}$  were available for

dispensing (table 2). Competitor R only offers 8 dispensing steps meaning a maximum dispensing volume of 2,000  $\mu\text{L}$  or 4,000  $\mu\text{L}$ , respectively. This was shown in our test as the total dispensing volume was 2,008  $\mu\text{L}$  and 4,011  $\mu\text{L}$ . 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 competitor B and R have less total dispensing volume and more residual volume than Combitips advanced.

Figure 4 illustrates the percentage distribution of the filling volumes for 2.5 mL tips using 250  $\mu\text{L}$  dispensing steps. For 5 mL tips, the same relationship was determined. A significant amount of liquid needed as reverse-, remaining stroke, and residual volume in both competitor tips was visible. If the liquid was not re-dispensed into the source vessel it was

wasted. This increased residual volume of sample liquid, reagent solution and raised costs as well as effort. In contrast using Combitips advanced combined with Multipette/Repeater M4 enabled usage of the full liquid filling while having only 5% total residual volume.

## Conclusion

It was shown that Combitips advanced only need 5-7% additional filling volume. The tested competitor dispenser tips 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 tested competitors. 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 Combitips advanced and Multipette/Repeater system.

**Ordering information**

Product description	Color coding	Order no.				
		Eppendorf Quality™	PCR clean	Sterile*	Biopur®	Forensic DNA Grade
Combitips advanced®						
0.1 mL	White	0030 089.405	0030 089.766	0030 089.510	0030 089.618	
0.2 mL	Light blue	0030 089.413	0030 089.774	0030 089.529	0030 089.626	
0.5 mL	Purple	0030 089.421	0030 089.782	0030 089.537	0030 089.634	
1 mL	Yellow	0030 089.430	0030 089.790	0030 089.545	0030 089.642	0030 089.855
2.5 mL	Green	0030 089.448	0030 089.804	0030 089.553	0030 089.650	0030 089.863
5 mL	Blue	0030 089.456	0030 089.812	0030 089.561	0030 089.669	0030 089.871
10 mL	Orange	0030 089.464	0030 089.820	0030 089.570	0030 089.677	
25 mL	Red	0030 089.472	0030 089.839	0030 089.588	0030 089.685	
50 mL	Light gray	0030 089.480	0030 089.847	0030 089.596	0030 089.693	

\* only available in North America

**Accessories**

Adapter advanced						
25 mL, autoclavable, 1 piece	Red	0030 089.715				
25 mL, autoclavable, 7 pcs., individually wrapped	Red				0030 089.731	
50 mL, autoclavable, 1 piece	Light gray	0030 089.723				
50 mL, autoclavable, 7 pcs., individually wrapped	Light gray				0030 089.740	
<b>Combitips advanced® rack</b> , for 8 Combitips advanced® (0.1 – 10 mL)		0030 089.758				

Devices	Order no. international	Order no. North America
<b>Multipette®/Repeater® M4</b> , single-channel, 1 µL - 10 mL	4982 000.012	4982000012
<b>Multipette®/Repeater® E3</b> , single-channel, with charging cable and Combitips advanced® assortment pack (1 Combitip of each size), 1 µL – 50 mL	4987 000.010	4987000010
<b>Multipette®/Repeater® E3x</b> , single-channel, with charging cable and Combitips advanced® assortment pack (1 Combitip of each size), 1 µL – 50 mL	4987 000.029	4987000029

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