

# Userguide

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## Liquid volume required to fill Combitips advanced<sup>®</sup> completely, and comparison to the competition

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### Summary

**In order to fill a Combitip advanced completely, more liquid is required than indicated by its maximum value. Total filling volumes were measured to determine the actual filling volumes required for the Combitips advanced. These data led to recommendations regarding the volumes which should be provided to fill each Combitip advanced. In addition, positive displacement tips from two competitors were tested alongside the Combitips advanced, and the ratios between filling volume and maximum volume were analyzed.**

### Introduction

The manual hand dispenser Multipette plus (in North America: Repeater plus) and the electronic versions Multipette stream/Xstream (in North America: Repeater stream/Xstream), in combination with the Eppendorf Combitips advanced, form a complete system for dispensing applications in the laboratory.

Generally, the positive displacement system is operated as follows: following aspiration of the liquid, the dispensing lever (manual dispenser) or actuate key (electronic dispenser) must be pressed once in order to trigger the reverse stroke. Thus, the system is entering a defined starting position. Following the final dispensing step, the piston has not yet reached its lowest position, thus avoiding the dispensation of too small a volume during this final step. Hence, technical specifications require that a certain amount of residual liquid will remain inside the dispensing tip which may be discarded in a separate disposal step. Subsequently, the dispensing tip

may be removed or refilled.

The reverse stroke and discard step are not considered dispensing steps. Therefore, more liquid needs to be aspirated into the dispensing tip than will subsequently be distributed during the dispensing application. This fact needs to be considered when working with solutions which are available in limited quantities. The excess liquid may be dispensed back into the supply vessel or discarded.

In this Userguide, the extra volume which is required in addition to the maximum volume in order to fill the Combitip advanced entirely is determined using a standard application. The data obtained regarding these filling volumes form the basis for calculations of the total amount of solution which is required for any given application. Furthermore, the ratio between the filling volume and the volume actually dispensed by the Multipette plus manual system will be presented in comparison to manual systems by two competitors.

Material and methods

The Eppendorf Multipette plus and the Multipette stream (software version: 03.07.00) were used to determine the filling volumes of the Combitips advanced. For the Multipette stream, dispensing mode (DIS) was selected and the speed was set to level 7. All sizes of Combitips advanced in the volume range between 0.1 mL and 50 mL volume were tested (see Table 1).

To determine the relationship between the filling volume and total dispensing volume of the Combitips advanced and the dispensing tips from two competitors, these tips were used in combination with the corresponding mechanical/manual dispenser. The determination was carried out using 2.5 mL, 5 mL and 10 mL tips in selection dial positions 1 and 5.

In order to determine the filling volumes, the corresponding dispensing tip was filled with distilled water and the removed liquid was calculated gravimetrically. Subsequently, each dispensing step (blow out, total dispensing volume and discard stroke) was gravimetrically determined. The measured values were converted to volumes while taking the density factor Z for distilled water (dependent on temperature and atmospheric pressure) into account. The loss was determined based on the difference between the filling volume and dispensed volumes. The WXTS205DU (Mettler Toledo) balance was used for weighing.

Filling volume	Volume required to fill a dispensing tip completely; the sum of the volumes of the reverse stroke, all dispensing volumes, the discard stroke and the loss.
Reverse stroke	Following liquid aspiration, the piston is brought to a defined 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 dispensing volumes
Discard stroke	After all dispensing steps have been completed, the small amount of liquid which remains as a safety reserve will be dispensed as a discard stroke.
Maximum volume	The maximum usable volume for all dispensing steps
Travel range	The distance that a dispensing tip piston needs to be moved upward in order to fill a dispensing tip completely.

Results

As shown in Fig. 1, the average excess volume ranged between 5–8 % of the maximum volume for each Combitip advanced. The excess volume tends to be lower with the Multipette stream than when using the Multipette plus. These data reveal a relatively narrow range of variation of these values, thus confirming the high manufacturing quality of the Combitips advanced, as well as highlighting the fact that that the Combitip advanced and Multipette plus or stream/Xstream are a well-coordinated system.

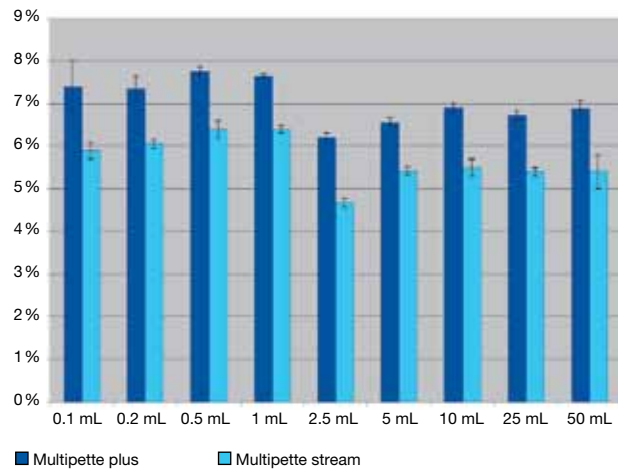


Fig. 1: Percentage of the additional amount of liquid required when using the Multipette plus and Multipette stream for any size Combitip advanced.

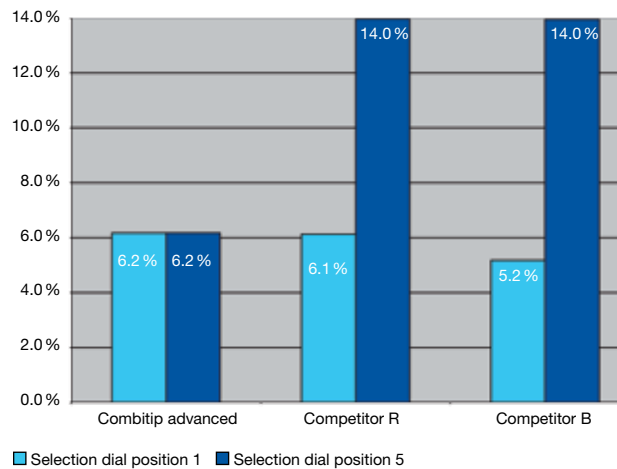
Table 1 lists the volumes required to fill any Combitip advanced entirely. This table represents a recommendation based on previous experience; it is therefore recommended to provide an additional 10 % of the maximum volume of the respective Combitip. This information is helpful when estimating whether a planned dispensing procedure can be carried out with a given amount of liquid.

Combitip advanced	Extra volume	Volume required for complete filling
0.1 mL	10 µL	110 µL
0.2 mL	20 µL	220 µL
0.5 mL	50 µL	550 µL
1 mL	100 µL	1.1 mL
2.5 mL	250 µL	2.75 mL
5 mL	500 µL	5.5 mL
10 mL	1000 µL	11 mL
25 mL	2.5 mL	27.5 mL
50 mL	5.0 mL	55.0 mL

**Tab. 1:** Extra volume to be provided and total volume required for complete filling.

For the manual hand dispenser Multipette plus the travel range required for complete filling, plus the reverse stroke, are identical for every position chosen on the selection dial. Therefore, the filling volume is independent of the dispensing volume. For competitors R and B, the reverse stroke depends on the selection dial position. Furthermore, for competitor R, also the travel range is dependent on the position of the selection dial; the dispensing volume selected will thus change the filling volume.

As shown in Fig. 2, the comparison between filling volumes and their respective dispensing volumes illustrates the fact that the competitors' dispensing tips, when set to selection dial position 1 (the competitors' smallest possible dispensing volume) have a slightly smaller filling volume

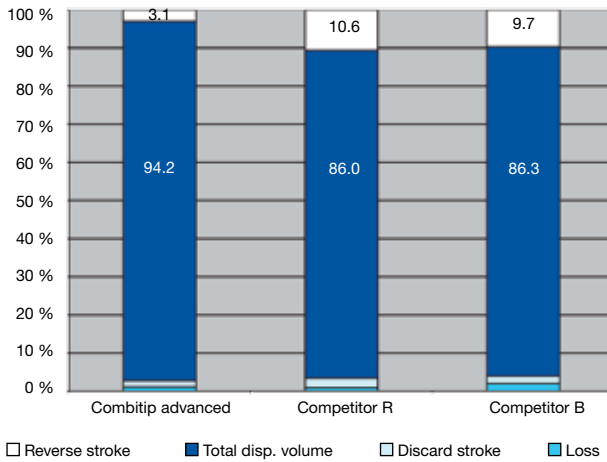


**Fig. 2:** Extra volume required, expressed in percent in relation to the total dispensing volume, and relationship to the position of the selection dial.

than the Combitips advanced used in combination with the Multipette plus. In contrast, when set to selection dial position 5, the competitors' largest possible dispensing volume, the filling volume required to fill their respective dispensing tips, is significantly larger.

Competitor R only offers a total of 5 settings for the dispensing volume and competitor B offers 9 settings, whereas the Multipette plus features 20 settings for the dispensing volume. Although selection dial settings 1 and 5 correspond to the same dispensing volumes on the competitors' instruments, these settings represent neither the smallest nor largest dispensing volumes. While the competition can provide only 48 dispensing steps in selection dial position 1, the Multipette plus features 50 dispensing steps. In selection dial position 5, ten dispensing steps are available on the Multipette plus while competitor B offers 9 steps in this setting and competitor R only offers 8 dispensing steps.

The dispensing volume was gravimetrically analyzed in selection dial position 5 in order to determine the relationship between aspirated and dispensed volumes. Figure 3 illustrates the percentage distribution of the filling volumes for 2.5 mL dispensing tips. For 5 mL and 10 mL tips, the same relationship was determined for the corresponding components of the filling volume.



**Fig. 3:** Percentage distribution of the filling volumes for 2.5 mL dispensing tips.

Table 2 shows the average value, calculated from the absolute values. This table clearly illustrates the differences between the total dispensing volume and the dispensing tip sizes specified by the manufacturer in the competitors' instruments. Altogether, a smaller volume is dispensed than one would expect based on the respective size specification. The dispensing volume does not match the size specification of the dispensing tip.

In contrast, for the Combitips advanced, the total dispensing volume is in accordance with the specified size of the dispensing tip. For competitor R, even the filling volume is below the dispensing tip size specified by the manufacturer. Taken together, when the Combitips advanced are compared to the competitors tested, one may conclude that the Combitips advanced require more volume for complete filling of the dispensing tip; however, for the Combitips advanced, the dispensing volume of a given size is in agreement with the nominal volume of the tip, which is not the case for the competition.

	Combitip advanced	Competitor B	Competitor R
<b>2.5 mL</b>			
Filling vol. µL	2655	2600	2349
Dispensing vol. µL	2500	2244	2019
<b>5 mL</b>			
Filling vol. µL	5328	5210	4686
Dispensing vol. µL	5000	4480	4028
<b>10 mL</b>			
Filling vol. µL	10690	10460	9360
Dispensing vol. µL	9993	8980	8066

**Tab. 2:** Relationship between filling volume and total dispensing volume for 2.5 mL, 5 mL and 10 mL dispensing tips.

## Ordering information

Name		Order no.	Order no.	Order no.	Order no.
Combitips advanced®	Color coding	Eppendorf Quality	PCR clean*	Sterile**	Eppendorf Biopur®
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
2.5 mL	Green	0030 089.448	0030 089.804	0030 089.553	0030 089.650
5 mL	Blue	0030 089.456	0030 089.812	0030 089.561	0030 089.669
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
Accessories					
25 mL adapter (1 pc.)	Red	0030 089.715			
50 mL adapter (1 pc.)	Light gray	0030 089.723			
25 mL adapter (7 pcs.)	Red				0030 089.731
50 mL adapter (7 pcs.)	Light gray				0030 089.740
Combitip Rack (for 8 Combitips advanced, 0.1 mL–10 mL)		0030 089.758			

Name	Order no. International	Order no. North America
Multipette plus®/Repeater® plus	4981 000.019	022260201
Multipette stream®/Repeater® stream	4986 000.017	022460803
Multipette Xstream®/Repeater® Xstream	4986 000.025	022460811

\*Not available in North America

\*\*Only available in North America


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Application Support Europe: Tel: +49 1803 666 789 (Preis je nach Tarif im Ausland; 9 ct/min aus dem dt. Festnetz; Mobilfunkhöchstpreis 42 ct/min)

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