APPLICATION NOTE No. 386

Precise and Accurate whole Blood Dispensing with Multipette® E3x and Combitips advanced®

Vincent Dufey[1], Muriel Art[1], Hanaë Henke[2]
[1]Eppendorf Application Technologies S.A., Namur, Belgium
[2]Eppendorf AG, Hamburg, Germany

Abstract

Dispensing of whole blood samples is challenging and needs careful consideration of the appropriate pipetting tool. We showed that using a positive displacement system, such as the Eppendorf Multipette E3x, is suitable for dispensing whole blood samples. Compared to an electronic air cushion pipette the results obtained with the Multipette E3x were accurate and precise. Furthermore, the Multipette E3x and Combitips advanced system was compared to two competitor instruments. This evaluation led to the conclusion, that one competitor instrument already failed to meet the supplier´s specifications when dispensing water. Therefore, only the second competitor instrument was tested with whole blood. This instrument showed deviation from the manufacturers precision limits. The only instrument within supplier´s specifications when dispensing whole blood was the Multipette E3x.

Introduction

During the past 40 years, liquid delivery by pipetting became a common laboratory process. To fulfill this routine task, the researcher can select the right device among instruments operating according to two different dispensing principles: via air cushion or positive displacement [1]. In both cases liquid transfer is realized by a piston that moves within a cylinder. In an air cushion pipette, air is present between the aspirated sample and the piston inside the pipette. In a positive displacement instrument the piston is directly integrated in the consumable. Consequently, no compressible air cushion is present between the piston and the sample liquid in those instruments. In this case, the sample is in direct contact with the piston like in a syringe.

The presence of this air cushion clearly differentiates both kind of instruments. Being elastic, the air cushion can be significantly influenced by environmental parameters (relative humidity, room temperature, air pressure) as well as by physical properties of the pipetted liquid (density, viscosity, liquid temperature, vapor pressure) [2]. For this reason, and especially when accurate pipetting is crucial for the application, air cushion instruments should only be used for handling aqueous solutions.

Having no elastic air cushion, a positive displacement pipette is not affected by the characteristics of the sample or laboratory conditions. Therefore, those instruments are ideal for dispensing all complex liquids having physical properties different than water.

Whole blood is particularly complex. This biological fluid is composed of plasma and cells (red blood cells, white blood cells and platelets). Whole blood also contains a significant amount of proteins like albumin and globulins. All those components make blood denser and more viscous than water. As several applications (forensic analysis, blood cell culture, RNA/DNA extraction, etc.) require exact and reproducible blood pipetting, the appropriate dispensing system has to be carefully selected.

In this study, we compared an electronic positive displacement instrument, the Multipette E3x, to the Eppendorf Xplorer® plus, a more commonly used electronic air cushion pipette. We demonstrated that a positive displacement system is the most appropriate solution to handle whole blood accurately and precisely. The Eppendorf dispenser was also compared to two major competitors pointing out the impact of high quality instruments and excellent instrument-consumable association on the application.

Materials and Methods

Materials

Instruments

> Eppendorf Xplorer plus, electronic pipette, 50–1000 µL (order no. 4861 000.732)
> Eppendorf Xplorer plus, electronic pipette, 0.5–10 mL (order no. 4862 000.759)
> Eppendorf, Multipette E3x (order no. 4987 000.029)
> Competitor B, electronic dispenser
> Competitor R, electronic dispenser

Consumables

> Eppendorf, epT.I.P.S.® standard 50–1000 µL (order no. 0030 000.927)
> Eppendorf, ep T.I.P.S. standard 0.5–10 mL (order no. 0030 000.765)
> Eppendorf, Combitips advanced 1 mL standard (order no. 0030 089.430)
> Eppendorf, Combitips advanced 10 mL standard (order no. 0030 089.464)
> Competitor B, dispenser tips, 1 mL and 10 mL
> Competitor R, dispenser tips, 1 mL and 10 mL

Instrument Calibration

> Mettler-Toledo®, micro balance Excellence plus XP26PC (Mettler-Toledo, Ohio, USA; order no. 11106021)
> Sartorius®, Analytical balance Cubis® MSE224S-100-DA (Sartorius, Göttingen, Germany; order no. MSE-224S-100-DA)
> VWR, Water Molecular Biology Grade (VWR International, Pennsylvania, USA; order no. 733-1090)
> Human whole blood (pool of 4 blood samples)

Methods

Blood density determination

The density of human blood used in this study was determined by using a pycnometer of 10 cm^3 (BRAND® BLAUBRAND®; BRAND, GmbH + Co KG, Wertheim, Germany; order no 43308). Also called density bottle, this calibrated device is provided with a certificate notifying its exact capacity when filled with water at a specific temperature (20 °C). Here, the exact volume was 9.8422 cm^3.
To determine the blood density, the pycnometer was filled with the liquid of interest and weighed on an analytical balance. From the volume (specified on the certificate) and the measured mass, the density was determined. The value obtained (1.058 kg/liter) was used to determine the Z factor.

Imprecision and inaccuracy determination by gravimetric analysis

Systematic and random errors were determined using gravimetric methods in accordance with the EN ISO 8655 standard [3]. As requested by the norm, tests were carried out in a draught-free room. During testing, relative humidity was above 50 % and temperature was constant. Instruments, consumables and sample liquids were equilibrated to the test room for at least 2 hours before starting the test. For determining errors, the sample liquid was dispensed ten times into a vessel and weighed. For each condition, three series of ten pipetting/dispensing steps were performed. A new consumable was used for each series. The systematic error (inaccuracy) and the random error (imprecision) were determined for each series of 10 measurements. Three values were obtained per condition, from which the average and standard deviation were calculated.

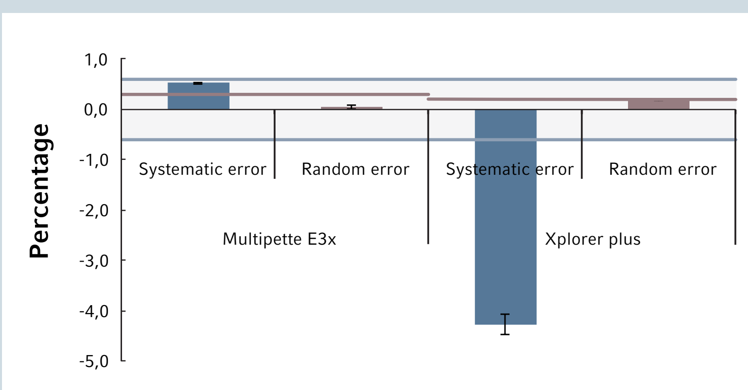
Results and Discussion

Selection of the right dispensing system
Accuracy and precision of a liquid handling instrument are defined with water and can only be guaranteed with this liquid. When the physical properties of the used liquid differ from water, this can highly impact pipetting performances and have to be taken into account when selecting a dispensing system.
Human blood density depends on the proportion of its components, in particular the number of red blood cells and the amount of protein.
For this reason, the blood density is higher than water density (1.000 kg/liter). The density of blood used in this study was experimentally determined to be 1.058 kg/liter. Blood is also more viscous than water. Value of blood viscosity is 4.5 Pa/s on average. Whereas water has a viscosity of 1 Pa/s.
In order to determine the most appropriate dispensing system to deliver an accurate and precise volume of human whole blood, air cushion pipettes were compared to a positive displacement instrument. To minimize operators' influence, electronic instruments were preferred.

Two air cushion Xplorer plus pipettes with a maximum volume of 1 mL and 10 mL were used in combination with the appropriate tips (1 mL and 10 mL). As positive displacement instrument, the Multipette E3x, was used in combination with Combitips advanced 1 mL and 10 mL. Pipetting and dispensing were evaluated for both systems.

Pipetting mode
As described by Ewald in the Eppendorf Userguide No. 21 [2], when used in pipetting mode, an air displacement pipette should deliver smaller volumes of high density liquids as compared to aqueous solutions. This theory was confirmed with whole blood. As shown in figures 1 A and 1 B, independent of the volume tested, the volume delivered by the Xplorer plus pipette is significantly less than expected inducing the instrument accuracy not being in compliance with the technical limit. On the opposite, by using a positive displacement system, an accurate pipetting of blood is feasible within the systematic error limits (0.52 % and 0.26 % respectively).

1 A



1 B

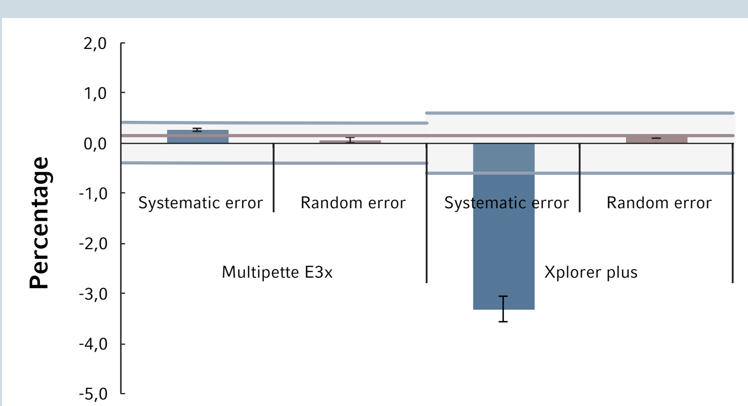


Figure 1: Results of blood pipetting using Multipette E3x and an air cushion pipette. Systematic error (blue) and random error (rose) for 10 x 1 mL (A) and 10 x 10 mL (B) are compared to technical specifications (n=3).

The instrument precision is not affected by the liquid density. This was demonstrated by 10 reproducible indvidual values as displayed in figure 2 A and B.

With random error values below 0.2 %, both instruments generated a random error lower than the acceptable error limit.

2 A

2 B

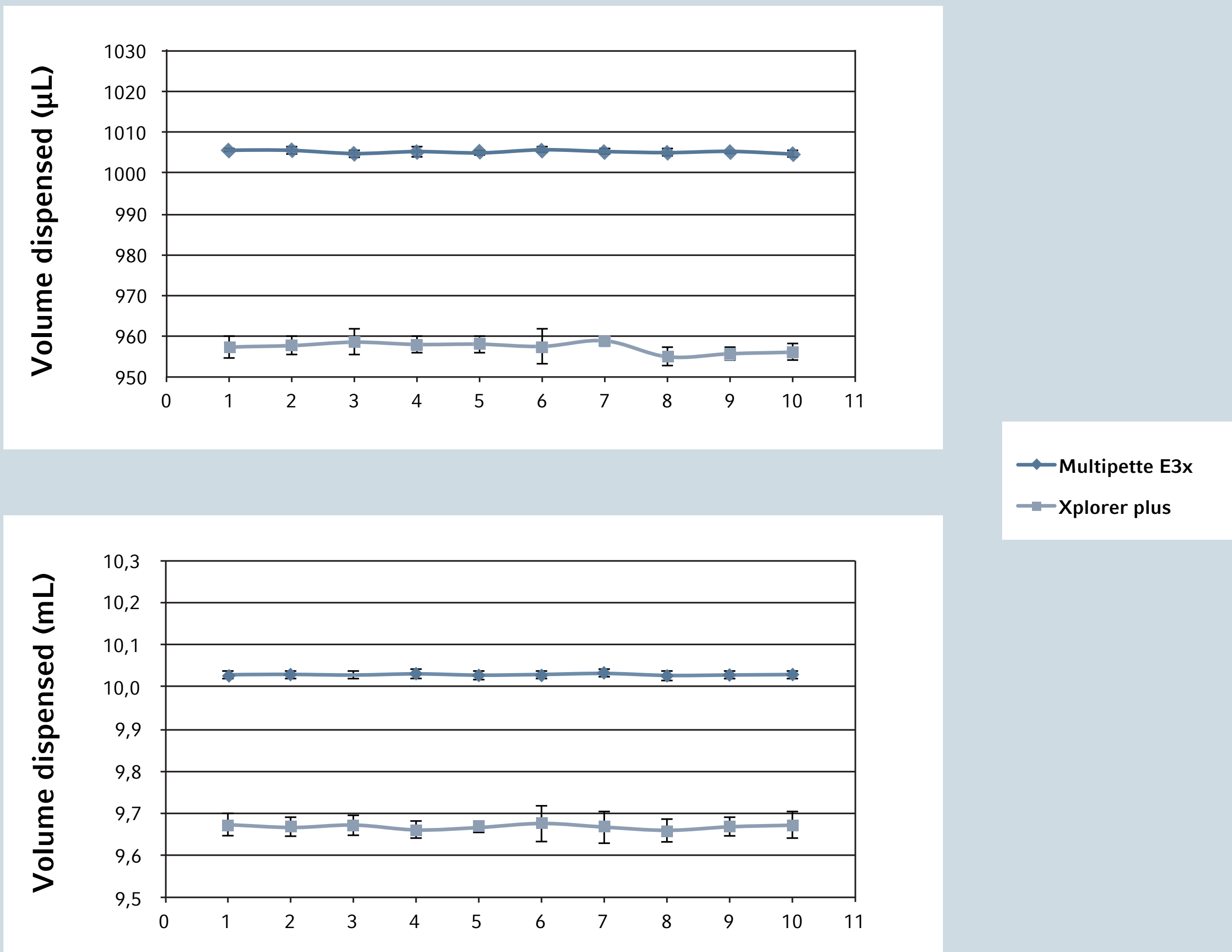


Figure 2: Results of blood pipetting using Multipette E3x and an air cushion pipette.
Individual values for 10 x 1 mL (A) and 10 x 10 mL (B) (n=3).

Dispensing mode

Data obtained for dispensing mode is shown in Figure 3. Again, systematic errors obtained with the air cushion pipette (1.52 % and 1.62 % respectively) are significantly higher than the errors obtained with the dispenser (0.45 % and 0.39 % respectively). In this case, the volume delivered by the air cushion pipette is higher than expected opposing data obtained from pipetting mode. This could be attributed to the reduced dead volume. The tip is filled completely to dispense the volume (100 µL or 1 mL) in multiple steps leading to a small air cushion. While in pipette mode 100 µL or 1 mL were aspirated into the tip leading to a bigger air dead volume above the liquid.

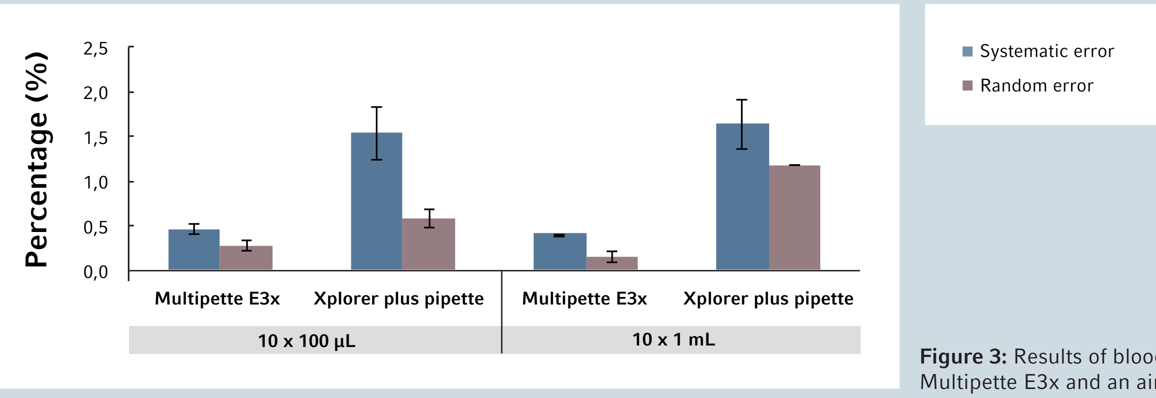


Figure 3: Results of blood dispensing using Multipette E3x and an air cushion pipette (n=3).

Besides an accurate blood dispensing, the positive displacement instrument also permits a reproducible liquid delivery (random errors of 0.26 % and 0.14 % respectively), whereas the air cushion system is more variable (random errors of 0.56 % and 1.16 % respectively).

As shown by the individual values in Figure 4, the dispensing variability is particularly prominent when a larger volume is dispensed. In this case, the instrument precision is even above the acceptable error limit.

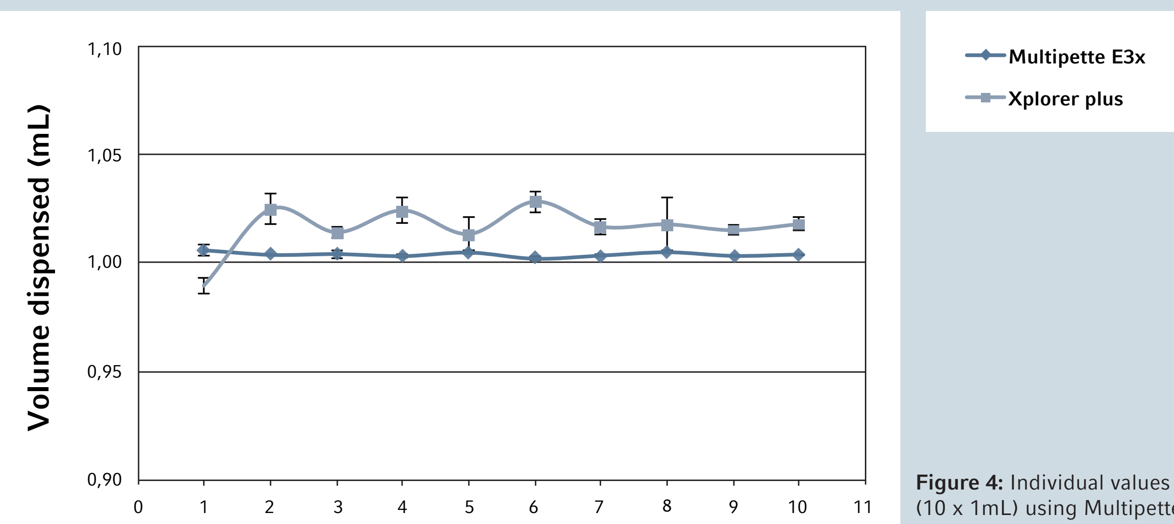


Figure 4: Individual values of blood dispensing (10 x 1mL) using Multipette E3x and an air cushion pipette (n=3).

Comparing both liquid handling systems, the results indicate that a positive displacement instrument is the right choice when accurate and precise dispensing of whole blood is required. Indeed, this dispensing system is not impacted by the blood's physical properties independent of the selected operating mode. It delivers exact and reproducible volumes even in the high volume range.

Selection of the most reliable dispenser

Knowing that a positive displacement system is the best option to guarantee a precise and accurate dispensing of complex solutions such as blood, customers have to select their instrument carefully. As dispenser tips are specifically designed to be used with its dedicated instrument, dispenser and consumable have to be considered as an inseparable combination. Indeed, with a positive displacement system the accuracy depends on the disposable tip quality, even more than with an air cushion pipette. In order to determine the most reliable equipment to dispense whole blood, the Eppendorf Multipette E3x was compared to two major competitors: dispenser B and dispenser R. Each instrument was used with their own consumables and was tested in both operating modes (pipetting and dispensing).
As shown on tables 1 and 2, accuracy and precision claimed by the supplier are not always reached, even when the dispensed liquid is water. In pipetting mode as well as in dispensing mode, the dispenser R combined with its dedicated 10 mL dispenser tips generates a systematic error above the upper limit defined by the supplier. In contrast the Eppendorf Multipette E3x and the dispenser B deliver accuracy within declared specifications.

Table 1 and 2: Accuracies and precisions obtained with three dispensers used in dispensing mode to deliver water. Values are compared to supplier specifications. Green highlighting indicates that the value is within the limits defined by the supplier. Red highlighting indicates that the value is outside the limits defined by the supplier (n=3).

Table 1

Pipetting Mode		Multipette® E3x		Dispenser B		Dispenser R	
Accuracy	Volume pipetted	Specification	Measured value	Specification	Measured value	Specification	Measured value
	10 x 1 mL	± 0,6%	0,24%	± 0,6%	-0,21%	± 0,8%	0,41%
	10 x 10 mL	± 0,4%	0,13%	± 0,4%	-0,07%	± 0,3%	0,59%
Precision	Volume pipetted	Specification	Measured value	Specification	Measured value	Specification	Measured value
	10 x 1 mL	≤ 0,3%	0,04%	≤ 0,3%	0,05%	≤ 0,2%	0,02%
	10 x 10 mL	≤ 0,15%	0,02%	≤ 0,15%	0,01%	≤ 0,2%	0,12%

Table 2

Dispensing Mode		Multipette® E3x		Dispenser B		Dispenser R	
Accuracy	Volume dispensed	Specification	Measured value	Specification	Measured value	Specification	Measured value
	10 x 100 µL	± 0,9%	0,25%	± 1,0%	-0,11%	± 0,8%	0,20%
	10 x 1 mL	± 0,5%	0,16%	± 0,7%	-0,09%	± 0,25%	0,64%
Precision	Volume dispensed	Specification	Measured value	Specification	Measured value	Specification	Measured value
	10 x 100 µL	≤ 0,55%	0,21%	≤ 0,8%	0,34%	≤ 0,55%	0,26%
	10 x 1 mL	≤ 0,25%	0,10%	≤ 0,8%	0,17%	≤ 0,5%	0,26%

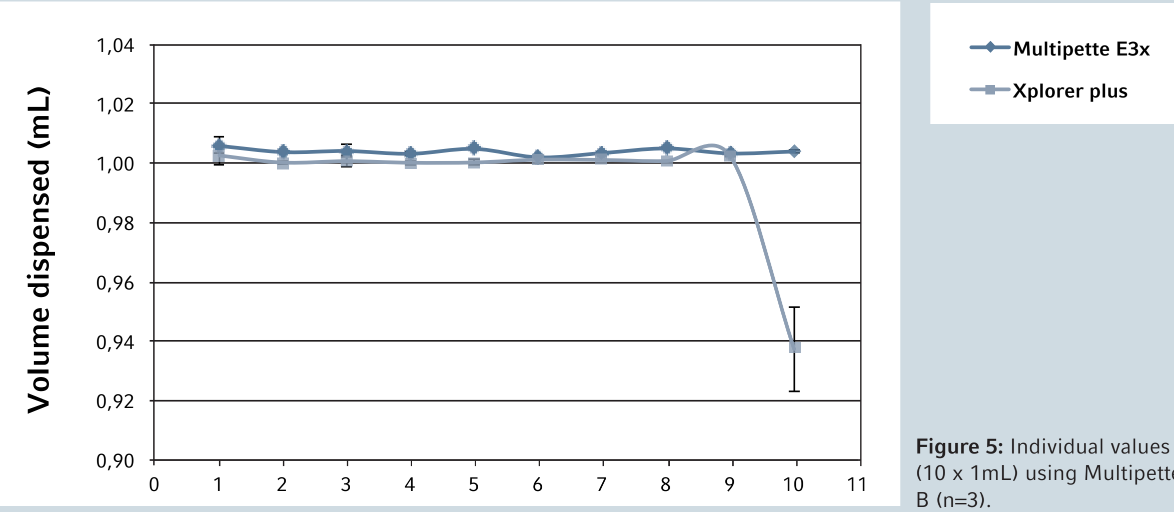


Figure 5: Individual values of blood dispensing (10 x 1mL) using Multipette E3x and dispenser B (n=3).

Tested with water, the Eppendorf Multipette E3x and the dispenser B were the most reliable systems. Consequently, only those instruments were afterwards evaluated for whole blood handling.

As mentioned previously, besides the instrument quality, the consumable itself has to be taken into account. During dispensing, viscous liquids can leave a film on the walls of the tip. This may induce a large volume remaining inside the tip. Air bubbles can also be created, reducing the liquid volume. When dispenser B combined with its 10 mL dispenser tips is used to dispense whole blood, this phenomenon is observed. This results in an aspirated blood volume insufficient to guarantee 10 successive reproducible dispensing steps.

As a consequence the last volume dispensed is significantly lower than expected, as demonstrated in Figure 5.
This last dispensing step highly impacts the instruments' precision. As shown in Table 3, the random error of dispenser B is, in this case, significantly above the acceptable error limit defined by the supplier. This phenomenon is also observed with the dispenser B 1 mL tips. However using those tips this effect is less promiment, allowing the instrument to stay just below the defined precision limit.

For an exact and reproducible blood dispensing, the Eppendorf Multipette E3x qualifies as the only option. Only this instrument, combined with Combitips advanced, ensures repeated reproducible blood dispensing in every tested case.

Table 3: Accuracies and precisions obtained with two dispensers used in dispensing mode to deliver whole blood. Values are compared to supplier specifications. Green highlighting indicates that the value is within the limits defined by the supplier. Red highlighting indicates that the value is outside the limits defined by the supplier (n=3).

Pipetting Mode		Multipette® E3x		Dispenser B	
Accuracy	Volume pipetted	Specification	Measured value	Specification	Measured value
	10 x 100 µL	± 0,9%	0,45%	± 1,0%	-0,16%
	10 x 1 mL	± 0,5%	0,39%	± 0,7%	-0,53%
Precision	Volume pipetted	Specification	Measured value	Specification	Measured value
	10 x 100 µL	≤ 0,55%	0,26%	≤ 0,8%	0,77%
	10 x 1 mL	≤ 0,25%	0,14%	≤ 0,8%	2,02%

Conclusion

Liquids having physical properties different from water are difficult to dispense and clearly affect pipette accuracy as well as precision. Having a higher density and viscosity, whole blood is a liquid very different from water. Positive displacement instruments, based on a direct contact between liquid and piston, should not be affected by liquid properties and should be the consequent choice to handle complex solutions such as blood. Therefore, the Eppendorf Multipette E3x, a positive displacement dispenser, has been compared to a classic air displacement pipette (Xplorer plus electronic pipette) to handle whole blood.
With an air cushion pipette the systematic error increases significantly, using both pipetting and dispensing mode. Depending to the operating mode selected, the volume delivered can be either smaller or higher than expected. At the opposite, the Multipette E3x performance stays stable in all circumstances ensuring an accurate and precise blood multi-dispensing. By comparing both liquid handling systems, this study confirms that a positive displacement system is the right instrument to be employed when exact and reproducible dispensing of blood is critical.
In this case, customers should select their instrument carefully among all options available on the market.

To determine the most reliable dispenser, the Eppendorf Multipette E3x was compared to two major competitors: dispenser B and dispenser R. We firstly demonstrated that even when the liquid dispensed is water, accuracy and precision claimed by the supplier are not always reached.
In pipetting mode as well as in dispensing mode, dispenser R combined with its dedicated 10 mL dispenser tip reproducibly generates a systematic error above the limit defined by the supplier. For dispensing blood, an instrument of good quality alone is not sufficient. Indeed, the consumable itself has to be considered as well. With dispenser B associated with its own consumable, the aspirated blood volume is insufficient to guarantee 10 consecutive reproducible dispensing steps. When the last volume dispensed is significantly lower than expected, the instrument precision can be outside acceptable limits defined by the supplier. For an exact and reproducible blood dispensing, the Eppendorf Multipette E3x is the right choice. As a matter of fact, this instrument comparison demonstrated, that among all dispensers tested only the Eppendorf Multipette E3x, combined with Combitips advanced, reaches accuracy and precision specifications in every case.

Literature

[1] Ewald K. Fundamentals of dispensing. Eppendorf Userguide N° 19, 2015. www.eppendorf.com

[2] Ewald K. Influence of physical parameters on the dispensed volume of air-cushion pipette. Eppendorf Userguide N° 21, 2015. www.eppendorf.com

[3] EN ISO 8655, Parts 1–6: Piston-operated volumetric apparatus. © ISO 2002.

Ordering Information		
Description	**Order no. international**	**Order no. North America**
Multipette® E3/ E3x, with charging cable and 2 Combitips advanced® assortment packs, 1 µL - 50 mL	4987 000.029	4987000029
Eppendorf Xplorer® plus, single channel, 50 - 1,000 µL	4861 000.732	4861000732
Eppendorf Xplorer® plus, single channel, 0.5 - 10 mL	4862 000.759	4862000759
epT.I.P.S.®, 50 - 1,000 µL, Eppendorf Quality™	0030 000.927	0030000927
epT.I.P.S.®, 0.5 - 10 mL, Eppendorf Quality™	0030 000.765	0030000765
Combitips advanced®, 1.0 mL, Eppendorf Quality™	0030 089.430	0030089430
Combitips advanced®, 10 mL, Eppendorf Quality™	0030 089.464	0030089464

Your local distributor: www.eppendorf.com/contact
Eppendorf AG · 22331 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com