Applications

Note 242 | September 2011

Technical Report

Exact dispensing of whole blood using the Eppendorf positive displacement system Multipette Xstream®/Repeater® Xstream

Natascha Weiß[1], Wei Sze Heng[2], Foong Teng Lu[2]
[1]Eppendorf AG, Hamburg, Germany; [2]Eppendorf Asia Pacific Headquarters, Kuala Lumpur, Malaysia

Abstract

Air cushion pipettes are of limited use for liquids whose properties differ significantly from water. This also pertains to whole blood and serum, as these fluids are denser and more viscous than water, and their composition is complex. Comparisons between an electronic air cushion pipette and an electronic positive displacement system, the Multipette/Repeater Xstream, show that much more accurate dispensing of blood is achieved in pipetting mode as well as dispensing mode. Thus, the Multipette/Repeater Xstream, in combination with Combitips plus, is capable of pipetting and dispensing blood virtually as accurately as water.

Introduction

Dispensing systems in the laboratory can function either by employing the air cushion principle, or they constitute a positive displacement system [1]. In an air cushion pipette, an air cushion separates the liquid in the plastic tip from the piston inside the pipette. Since this air cushion is elastic, it is strongly influenced by the properties of the sample, such as density, vapor pressure, viscosity and temperature, as well as partly by environmental conditions (temperature, relative humidity, air pressure) [2]. Generally, air cushion pipettes are adjusted to distilled water, allowing aqueous solutions commonly used in the laboratory to be dispensed with high accuracy and precision. When samples whose properties differ significantly from those of water are used, air cushion systems reach their limit, even when pipetting techniques such as reverse pipetting or an adjustment of the pipette offer options to improve dispensing results [3].

A positive displacement system is characterized by a piston which is integrated in the plastic tip and therefore has direct contact with the sample (like a syringe). The piston ensures positive displacement of the liquid to be dispensed with a minimal air cushion [1]. In this way, the physical properties of the liquid have very little influence on the volume of the liquid to be aspirated or dispensed. Thus, this system is ideally suited for dispensing of organic solvents with high vapor pressure, viscous solutions [4] or foaming solutions. Simultaneously, the integrated piston prevents contamination from aerosols.

Whole blood and serum are very different from water. They display higher viscosity and density, and they contain proteins which can wet the surface of the tip and cause foaming. Furthermore, blood, due to the cells contained therein, constitutes a suspension. When an air cushion pipette is used, these properties may cause problems, especially when small amounts of blood are to be measured accurately in order to obtain correct and reproducible data from sensitive analysis methods. This pertains to, for example, immunophenotyping via flow cytometry or the detection of trace minerals in whole blood.

A study conducted by the German PTB (PTB is the national metrology institute providing scientific and technical services) revealed that the use of air cushion pipettes for dispensing human serum, especially small volumes thereof, led to deviations of approximately 5 % when compared to water, whereas positive displacement pipettes did not show a large difference (< 1 % deviation) [5]. Adjustment of the air cushion pipette to accommodate the difference in density between water and serum may minimize the deviations. However, one must take note that the labor involved in adjusting a pipette is considerable, and that the new adjustment is only valid for the specific volume set on the pipette. Hence, an adjustable-volume pipette, in effect, becomes a fixed-volume pipette.
This Technical Report will investigate the differences in accuracy and precision during pipetting and dispensing of blood, comparing an air cushion system to a positive displacement system.

Material and Methods

In order to minimize the operator's influence on, for example, the dispensing speed, electronic dispensing systems were chosen for this test. In addition, electronic systems offer several functions such as pipetting and dispensing in one instrument.

Three electronic air cushion pipettes with maximum volumes of 100 µL, 1000 µL and 10 mL were used, in combination with the appropriate tips of sizes 200 µL, 1000 µL and 10 mL. In parallel, the Multipette/Repeater Xstream, a dispenser utilizing the positive displacement principle, was used in combination with Combitips plus of sizes 0.1 mL, 1.0 mL and 10 mL.

Human whole blood served as sample material. Two different volumes were pipetted (100 µL and 1000 µL) and two different dispensing applications were performed (10 x 100 µL, 10 x 1000 µL). The systems tested are listed in Table 1. The systematic error (inaccuracy) and random error (imprecision) were determined for each set-up according to Eppendorf SOP [6]. For calculations, the density of blood (1.05 mg/µL) was taken into account.

For dispensing of solutions like blood using an air cushion pipette, reverse pipetting and pre-wetting of the tip are generally recommended. However, since these techniques are infrequently used in practice, they were not employed here; instead standard (forward) pipetting technique was chosen.

Table 1: Overview of the tested volumes and dispensing systems

Pipetting mode		
Volume	**100 µL**	**1000 µL**
Instruments + tips	Multipette/Repeater Xstream + Combitips plus 0.1 mL	Multipette/Repeater Xstream + Combitips plus 1.0 mL
	Air cushion pipette 100 µL + 200 µL pipette tips	Air cushion pipette 1000 µL + 1000 µL pipette tips
Dispensing mode		
Volumen	**10 x 100 µL**	**10 x 1000 µL**
Instruments + tips	Multipette/Repeater Xstream + Combitips plus 1.0 mL	Multipette/Repeater Xstream + Combitips plus 10 mL
	Air cushion pipette 1000 µL + 1000 µL pipette tips	Air cushion pipette 10 mL + 10 mL pipette tips

Results and Discussion

Pipetting mode

The results for pipetting mode are shown in Figures 1 A) and B). The accuracy for both volumes is considerably lower for the air cushion pipette (systematic error > 2.5 %), compared to the positive displacement dispenser Multipette/Repeater Xstream (systematic error < 0.1 %). The air cushion pipette does not conform to the technical data for distilled water when used for blood. The values obtained with the Multipette/Repeater Xstream are within the specifications for this experiment. The random error (imprecision) is similar for both instruments, with values below 0.2 %, which is lower than the respective error limits.

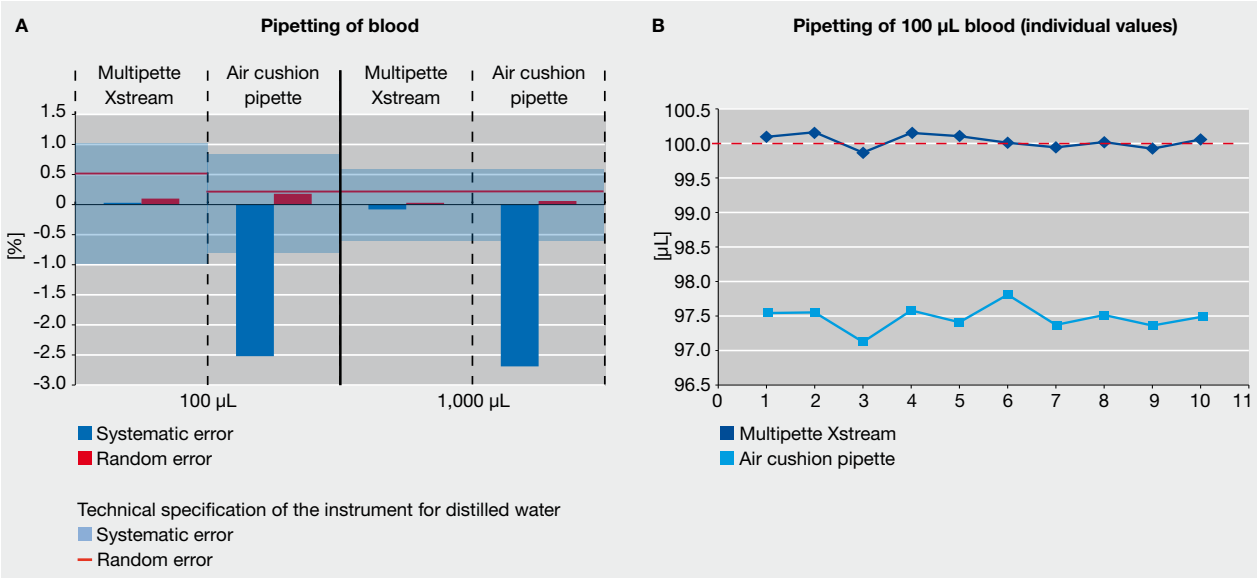

Figure 1: Results for pipetting of blood using Multipette/Repeater Xstream and an air cushion pipette.
A) Systematic and random error for 100 µL and 1000 µL
B) Individual values for 100 µL

Dispensing mode

The data obtained for dispensing mode are shown in Figures 2 A) and B). The systematic error of the air cushion system (3.23 % and 1.90 %, respectively) is again higher than that of the positive displacement system (0.11 % and 0.01 %, respectively). In this case the air cushion pipette dispensed, on average, too much blood whereas in the pipetting mode it was too little. This could be attributed to the longer tip size which is required for dispensing 10 times the same volume per dispensing step.

While the random error is also very low for the Multipette/ Repeater Xstream (< 0.15 %), these values are higher for the air cushion pipette at 0.76 % for 10 x 100 µL and 0.47 % for 10 x 1000 µL, respectively. The air cushion, which increases in size during the dispensing action, may play a role. In addition, a protein containing sample causes uneven wetting of the pipette tip during dispensing.

The actual error occurred during each dispensing step is illustrated by the individual measured values (Fig. 1 B and 2 B). For the air cushion pipette, the maximum deviations from the test volume are nearly 3 % during pipetting mode and almost 4.5 % during dispensing mode; these values are considerably higher than those obtained with the positive displacement system (below 0.2 % and 0.5 %, respectively).
Similar results as those obtained with blood are achieved when dispensing human serum (data not shown).

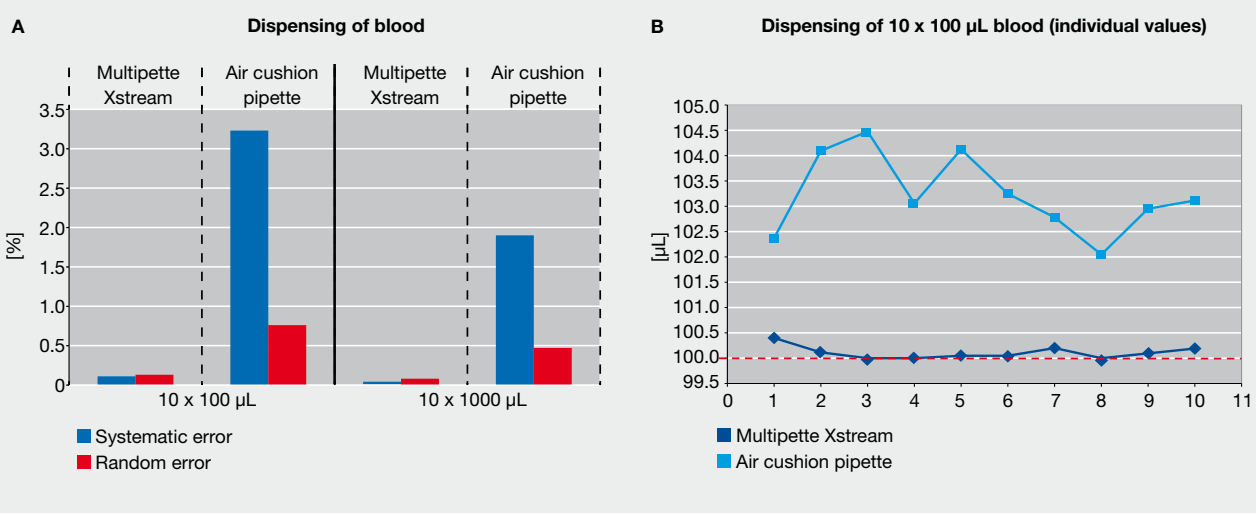

Figure 2: Results for dispensing of blood using Multipette/Repeater Xstream and an air cushion pipette
A) Systematic and random error for 10 x 100 µL and 10 x 1000 µL
B) Individual values for 10 x 100 µL

Techniques such as reverse pipetting and pre-wetting of the tip are generally recommended when viscous and protein-rich solutions are to be dispensed using an air cushion system. However, these techniques do not produce the same high accuracy and precision of a positive displacement system [4]. In addition, an air cushion pipette may be adjusted for dispensing blood in order to improve systematic error. However, one needs to take note that adjustment is time consuming and that it is only valid for the volume at which the pipette is set. An adjustment is, therefore, only practical for specific applications.

Conclusion

When accurate and precise dispensing of blood is critical, an air cushion pipette is of limited use. The systematic error falls outside the error limits defined for water and the individual measurements vary considerably, especially in dispensing mode. In contrast, the results obtained with the positive displacement system show only small deviations and they are well within the specifications. Thus, the Multipette/Repeater Xstream, in combination with the Combitips plus, is very well suited for exact dispensing of complex samples such as blood, an important prerequisite for accurate and reproducible analysis results.

References

[1] Userguide Liquid Handling 1/No. 19: Fundamentals of dispensing (www.eppendorf.com).
[2] Userguide Liquid Handling 3/No. 21: Influence of physical parameters on the dispensed volume of air-cushion pipette (www.eppendorf.com).
[3] Userguide Liquid Handling 2/No. 20: Impact of pipetting techniques on precision and accuracy (www.eppendorf.com).
[4] Eppendorf Application Note 211: Dispensing of highly viscous liquids (www.eppendorf.com).
[5] PTBnews 3/2000: Microlitre Dispensing of Human Blood Serum.
[6] Eppendorf SOP – Standard Operating Procedure for Pipettes (www.eppendorf.com).

Ordering information

Description	Order No. international	Order No. North America
Multipette Xstream®/Repeater® Xstream	4986 000.017	022460803
Multipette Xstream®/Repeater® Xstream	4986 000.025	022460811

Combitips plus®	Standard (set of 100)		Eppendorf Biopur® (individually wrapped, set of 100)	
	Order No. international	Order No. North America	Order No. international	Order No. North America
0.1 mL	0030 069.200	022265954	0030 069.404	022496000
0.2 mL	0030 069.218	022266004	0030 069.412	022496026
0.5 mL	0030 069.226	022266101	0030 069.420	022496042
1.0 mL	0030 069.234	022266209	0030 069.439	022496069
2.5 mL	0030 069.242	022266306	0030 069.447	022496085
5.0 mL	0030 069.250	022266403	0030 069.455	022496107
10 mL	0030 069.269	022266501	0030 069.463	022496123
25 mL	0030 069.293	022266551	0030 069.390	022496131
50 mL	0030 069.277	022266608	0030 069.471	022496140
Assortment pack Combitips plus®	0030 069.285	022266624	–	–

eppendorf
In touch with life

Your local distributor: www.eppendorf.com/worldwide

Eppendorf AG · 22331 Hamburg · Germany · Tel: +49 40 53801-0 · Fax: +49 40 538 01-556 · E-mail: eppendorf@eppendorf.com

Eppendorf North America, Inc. · 102 Motor Parkway · Hauppauge, N.Y. 11788-5178 · USA

Tel: +1 516 334 7500 · Toll free phone: +1 800-645-3050 · Fax: +1 516 334 7506 · E-mail: info@eppendorf.com

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)

E-mail: support@eppendorf.com

North America: Tel: +1 800 645 3050 · E-mail: techserv@eppendorf.com

Asia Pacific: Tel: +60 3 8023 6869 · E-mail: support_asiapacific@eppendorf.com

Order no.: AA242WW 020/GB1/WEB/0911/NEUH