MICROMAN® E: Exact and Secure Pipetting of Plasma and Whole Blood

APPLICATION NOTE AN0996

APPLICATION BENEFITS

Blood and plasma contain proteins that can wet the surface of a pipette tip and cause foaming. This makes them difficult to pipette precisely without contamination in point-of-care diagnostics applications.

SOLUTIONS

Comparisons between an air displacement pipette with tips, and a positive displacement pipette, MICROMAN® E with capillary pistons, show that the MICROMAN® E achieves more accurate dispensing of plasma and blood without contamination.

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INTRODUCTION

Some diseases can be detected by whole blood or plasma tests, which require microliter transfers. Whole blood and plasma are more viscous than water, and contain proteins that can wet the surface of the pipette tip and cause foaming. Furthermore, blood, due to the cells contained therein, constitutes a suspension. The analytical materials as a pipetting system must be adjusted to provide reliable results guaranteeing secure manipulation.

Dispensing systems in the laboratory can either employ an air displacement system, or a positive displacement system. In an air displacement pipette, an air cushion separates the liquid in the plastic tip from the capillary piston inside the pipette.

Like any gas, the air cushion between the piston and liquids interacts according to the characteristics of liquids (density, viscosity, volatility, presence of surfactants), as well as partially by lab environment (temperature variation, humidity). Viscous liquids, like whole blood with a viscosity six times more than that of water (approximately 5x10-3 Pa.S), characteristically flow in and out of a pipette tip slowly. If the tip is withdrawn too soon from the liquid reservoir, an air bubble forms in the tip, reducing the liquid volume. Additionally, whole blood or plasma may contaminate the internal pipette parts, as well as the user.

Conversely, with positive displacement pipettes the liquid does not come into contact with the pipette itself. Samples are instead aspirated and expelled through a capillary via a piston. Furthermore, the capillary piston isolates the aspirated sample from the inside the pipette by eliminating the air between the piston and sample. Thereby eliminating the risks of cross contamination that could affect results. There is no air cushion; therefore, the physical properties of the liquid have very little influence on the volume of the liquid to be aspirated or dispensed.

This application note demonstrates the advantages of using positive displacement pipettes, like Gilson's MICROMAN® E, over air displacement pipettes in diagnostic applications requiring exact and secure transfer of blood and plasma.

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MATERIALS & METHODS

The properties of viscous liquids and environmental factors can affect the accuracy and safety of air displacement dispensing. To replicate diagnostic laboratory situations, solutions of distilled water grade 3 (ISO 3696), EDTA pooled plasma (1.020 g/cm^3), and whole blood (1.039 g/cm^3) served as sample materials to show the impact of viscosity while pipetting.

For each test, 10 volumes were dispensed with an air displacement system—a standard pipette and associated tips - and with a positive displacement pipette - Gilson MICROMAN® E M50E and capillary pistons CP50. All measurements were taken by lab technicians pipetting all day in an immunodiagnostic testing systems company.

For dispensing of solutions like blood using an air displacement pipette, reverse mode pipetting is recommended. The purge stroke is used during preparation. During aspiration, an amount of liquid equal to the amount of purged air is added.

This amount compensates for the liquid that remains as film inside the tip during dispensing. Nevertheless, there is a loss of plasma and blood with this technique. A second technique is to use wide orifice tips, or cut the ends of the tips. This “lab trick” eases the liquid aspiration and dispensing for the standard pipette, thereby allowing the liquid to move more quickly through the tip end. These methods do not prevent residual sample left in the tip while dispensing. In this study, the standard forward mode was used.

Principle and Pipetting Mode Description

The forward mode is the standard aspirate and dispense mode (see Figures 1 and 2). In general, the precision of the forward mode relies on precise draining by air pressure (air displacement pipette) or internal wiping of the pipette barrel (positive displacement pipette).

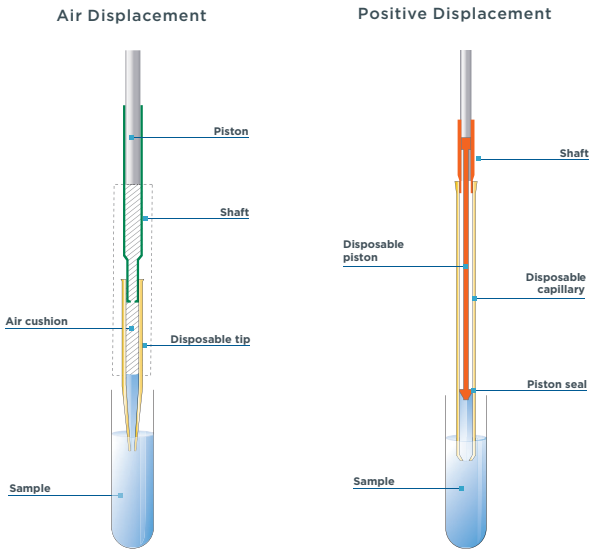

Figure 1
Two Pipetting Concepts—Air and Positive Displacement

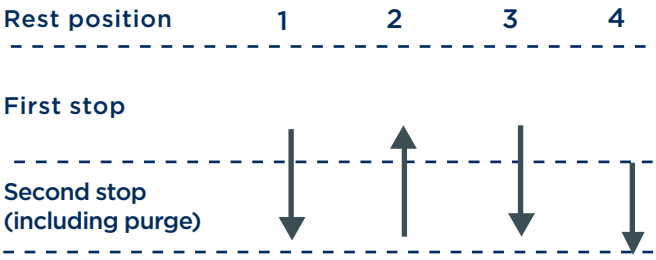

Figure 2
Forward Mode Pipetting

1. Preparation: Hold the instrument in a nearly vertical position. Depress the plunger smoothly to the first stop position.
2. Aspiration: Immerse the pipette tip in the liquid. Allow the plunger to move up smoothly to the rest position. Wait one second so that all the liquid has time to move up into the tip.
3. Distribution: Place the pipette tip at an angle (10° to 45°) against the inside wall of the receiving vessel. Depress the plunger smoothly to the first stop position.
4. Purge: Wait one second, and then depress the plunger to the second stop position. This “blow-out” stroke removes any remaining sample from the tip. Remove pipette tip end from sidewall by sliding it up the wall.

Forward mode with a positive displacement pipette is similar to the forward mode of air displacement pipette, but the purge step is replaced by the ejection of the capillary piston.

1. Preparation: Press the plunger button to the first stop. The piston moves to the appropriate position.
2. Aspiration: Immerse the capillary piston in the liquid. Release the plunger letting it move up to the home position. The piston moves up and the ambient pressure forces the desired volume of liquid through the orifice into the capillary.
3. Distribution: Press the plunger button to the first stop. The piston moves down and expels the liquid out of the capillary.
4. Ejection: Press the plunger all the way down to the second and last stop. Capillary and piston are ejected without hand contact.

Calculation Description
The average volume was determined by gravimetric measurements. The systematic error or inaccuracy of a pipette can be expressed as a percentage of the nominal volume:

$$E\% = (\bar{V} - Vo) \times 100 / Vo$$

E systematic error

Vo nominal volume

$\bar{V}$ mean volume

A percentage of random error or precision was calculated using this equation:

$$RSD = \frac{SD}{\bar{V}} \times 100$$

with

$$SD = \sqrt{\sum_{i=1}^{n} \frac{(\bar{V} - V_i)^2}{n-1}}$$

$$\bar{V} = \frac{1}{n} \sum_{i=1}^{n} V_i$$

RSD random error or relative standard deviation

SD standard deviation

V_i individually measured volume

n number of measurements

$\bar{V}$ mean volume

RESULTS AND DISCUSSION

In this study, when accuracy is necessary for dispensing blood solutions, a standard pipette is of limited use. The systematic error is 4–6 times greater than the MICROMAN® E, which shows only small deviation.

During calibration of pipettes, as shown in Figure 3, the systematic and random errors for pipetting 50 µL of water is similar for the standard pipette (0.72% and 0.05% respectively) compared to MICROMAN® E (-0.005% and 0.43%).

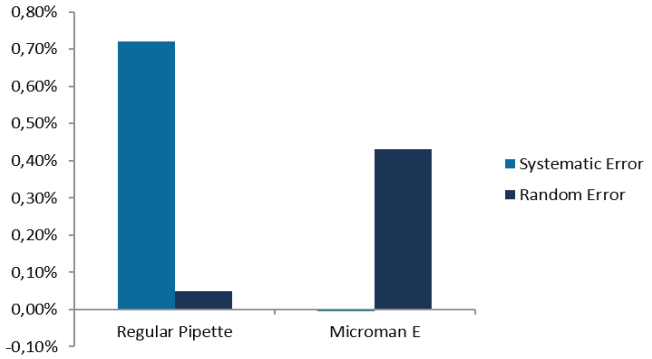

Figure 3
Systematic and random errors of water pipetting with MICROMAN® E versus standard pipette. Measurements were based on the average of ten gravimetric measurements per sample.

With blood and plasma, as shown in Figures 4 and 5, the systematic errors of the standard pipette (respectively plasma: -5.12% and blood: -7.25%) are higher than the ones of MICROMAN® E (plasma: -0.75% and blood: -0.79%).

MICROMAN® E is more accurate than standard pipettes when pipetting viscous liquids like plasma and blood.

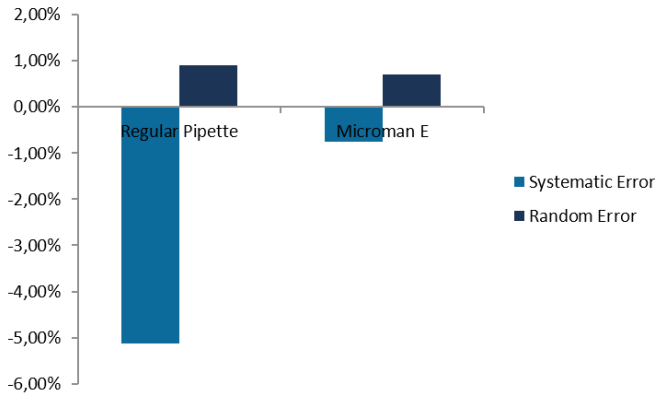

Figure 4
Systematic and random errors of plasma pipetting with MICROMAN® E versus standard pipette. Measurements were based on the average of ten gravimetric measurements per sample.

The standard pipette and MICROMAN® E have a similar repeatability whatever the liquids (between 0.7% and 1.0%).

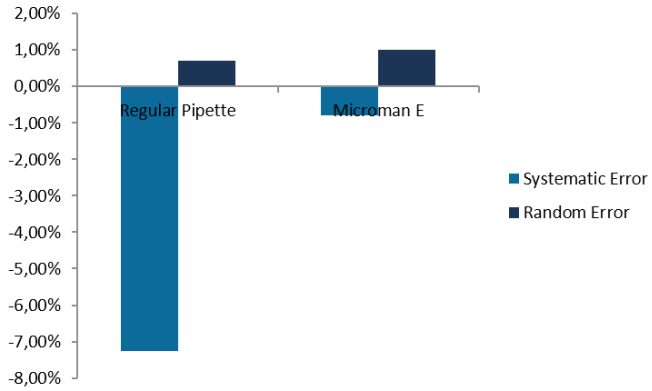

Figure 5
Systematic and random errors of blood pipetting with MICROMAN® E versus standard pipette. Measurements were based on the average of ten gravimetric measurements per sample.

With a positive displacement pipette accuracy and precision are guaranteed. Moreover, MICROMAN® E, positive displacement pipettes combined with disposable capillary pistons with built-in ejectors, offers clean and safe sample pipetting. Cross-contamination of nucleic acid samples for diagnostics studies is significantly reduced because disposable capillaries and pistons are used for each sample. Whole blood or plasma solutions can be safely pipetted without direct contact with the sample (Figure 1).

CONCLUSIONS

MICROMAN® E, a positive displacement pipette, with capillary pistons enables accurate measuring and dispensing of whole blood and plasma.

REFERENCES

1. Gilson SAS, 2014, Nadège BELHADJ, Achieve Precise Pipetting of Problem Liquids with MICROMAN® E
2. Gilson SAS, 2015, Guide to Pipetting