SAMPLE VOLUME METERING IN A DISPOSABLE MICROFLUIDIC CARTRIDGE

S. Vanhanen[1], P. Järvelä[2] and P. Kallio[1*]

[1]Department of Automation Science and Engineering, Tampere University of Technology, FINLAND and
[2]Department of Materials Science, Tampere University of Technology, FINLAND

ABSTRACT

This paper presents the results of a repeatability study of a developed sample volume metering system in a disposable microfluidic cartridge using human blood plasma samples (n=30). Repeatability is studied within and between patient samples. The metering concept utilizes passive valves and pressurized air to cut the sample plug to a desired volume on mass-produced thermoplastic cartridges. The cartridges are injection moulded of high impact polystyrene (PS-HI) and covered by laser welding a polystyrene (PS) plate on top of it. The results indicate coefficient of variation between the patients and within the patient samples to be 4.6% and 3.9%, respectively.

KEYWORDS: Sample Volume Metering, Disposable Microfluidic Cartridge, Blood Plasma, Repeatability

INTRODUCTION

Repeatable automated sample volume metering on a chip or on a cartridge provides significant benefits in point-of-care (POC) diagnostics. It increases speed, precision and cost-efficiency of POC diagnostics and also eliminates the need of the user's intervention and thus, offers an enlargement of point-of-care diagnostics from professional to patient use. Thereby, a patient is able to follow up such diseases at home which currently require visits at clinics. As another example, time-critical diagnosis of life-threatening conditions such as detection of troponin-I in myocardial infarction can be performed in the first aid instead of the more time-consuming procedures in central laboratories.

THEORY

Various microfluidic structures for sample volume metering using a passive valve and pressurized air have been published.[1][2][3] The cartridges are usually manufactured of glass [1] and polydimethylsiloxane (PDMS) [2][3]. The metered volumes of unknown samples are estimated by weight [1] or using machine vision [2]. In this work, we apply for the first time the passive valve-pressurized air concept on mass-produced thermoplastic cartridges and use a large number of patients and real patient samples.

EXPERIMENTAL

The manufactured cartridges are injection moulded of high-impact polystyrene (PS-HI) (Styrolux® 656C, BASF) using a hydraulic injection moulding machine Demag D 60-182 (N60 NCIII-K) model 1990. The injection moulded cartridge plate is covered with a commercial black polystyrene (PS) plate (Vikureen, Athlone Extrusion, Ireland). The cartridge plates are bonded by a laser transmission welding method using a contour welding technique and a fiber laser (λ=1090 nm). The distance of the weld is 150 µm ± 20 µm from the channel edge. Before the cartridges are sealed, both cartridge plates are washed with isopropanol and rinsed with deionized (DI) water. To avoid grease in microfluidic features, the cartridges are handled with gloves after washing until they are sealed.

The essential parts of the sample volume metering setup are a pressure regulator, a camera with a ring light, and a cartridge holder. The supply pressure is generated using a house-made pressure source, which is based on electrical to pressure (E/P) transducer T-2000 by Marsch Bellofram. The pressure source is controlled using a house-made LabVIEW based pressure control application, which utilizes a PCI-6229measurement card of National Instruments. The camera, Sony XCD-X710, is used with Macro Video Zoom Lens (MVZL) (18-108, F/2,5) manufactured by Optem. Successful imaging requires suitable illumination circumstances, which are created using the ring light. The ring light consists of white and red Luxeon Star LEDs of Lumileds. The block diagram of the test environment is illustrated in Figure 1.

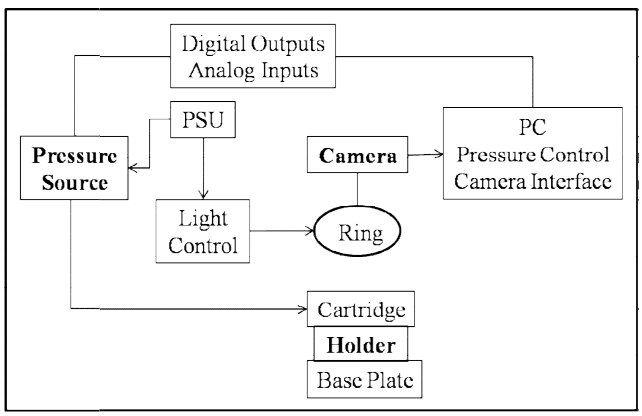

Figure 1: A block diagram of the measurement setup. The following abbreviations are used: power supply unit (PSU) and personal computer (PC).

The following aspects related to sample volume metering in immunoassay cartridges are discussed in this study: the repeatability of the volume metering within samples of one patient, the repeatability of the volume metering between several patients, and the adhesion of the patient samples on the cartridge materials and structures. In addition, the deviations in the dimensions of the injection moulded cartridges and in the surface tension of the samples are analyzed.

The volume metering sequence begins by injecting an unknown volume of a sample into the sample channel. Second, the sample is driven to the end of the sample channel, as presented in Figure 2 a), which is separated from a mixer using a passive valve. Last, the sample plug is cut using pressurized air as presented in Figure 2 b). The cut sample volume is estimated using images taken of the empty volume metering area and of the metered sample plug. The images are processed with common image processing algorithms using a MATLAB-based graphical user interface [4]. The volume of the sample plug is computed using the area covered by the liquid plug and the ideal three-dimensional channel geometry.

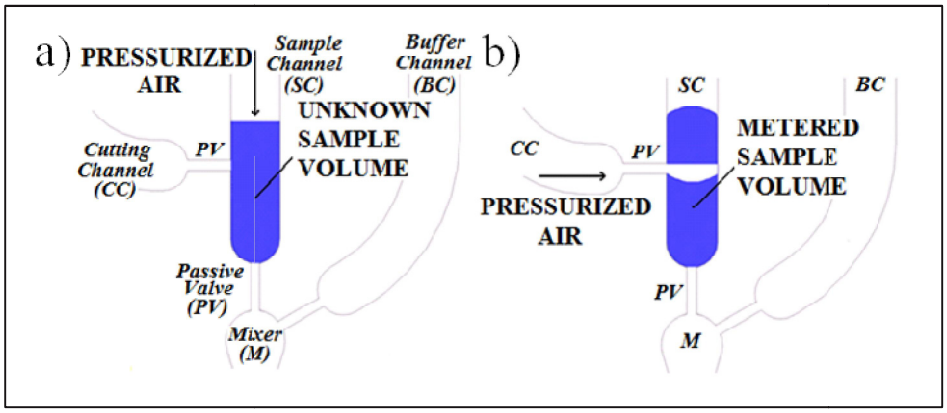

Figure 2: The volume metering area in a house-made immunoassay cartridge. a) The sample plug of an unknown volume in the volume metering area. b) The metered sample volume.

The sample adhesion on the cartridge plates and on the channel walls is measured by weighing each subsample three times using a laboratory scale (Precisa XR205SM-DR). The density value used for the plasma samples is ρ=1025 g/l. [5] The sample adhesion on the cartridge plates is studied by spreading a known volume of the sample on a known area and then removed from the surface by pouring. The sample adhesion on the channel walls is studied by injecting a known volume of the sample into the channel, stopped before the passive valve of the cutting channel (Fig. 2a), and finally, driven out of the channel using pressurized air.

The surface tension of the plasma samples is determined using Du Noüy tensiometer in order to find out the wetting properties of the samples. The deviations in the cross-section of the volume metering area are determined by cutting the cartridge and imaging the cross-section using scanning electron microscope (SEM).

The samples used in this study are plasma samples of human patients obtained from Finnish Research Tissue Bank (FinTiB) and Finnish Red Cross Blood Service (SPR Veripalvelu). The samples used in the volume metering measurements and adhesion studies are from FinTiB. They are collected from the sample line randomly from sample excess volumes. Samples are used within 24 hours from donation. A total number of 30 different patient samples are used in the volume metering measurements in which case each of them is divided into five subsamples in order to study the repeatability within each patient sample. The samples used in measuring the surface tension of plasma are from SPR Veripalvelu. The product is unsuitable for patient use, and sold with a code A0081V00. The samples have gone through the following treatment. After donation, whole blood is immediately cooled to a temperature range +17 – 24°C. The whole blood is separated as fresh, 2 – 23 hours after donation. The segregated plasma is frozen within 24 hours from donation. Gender, age, and blood type of all donators are unknown. During transport the samples are stored in a cellulose paper lined cool box with an ice bag. During the measurements each sample is taken out of the cool box in turn and the temperature is waited to become even.

RESULTS AND DISCUSSION

The repeatability results of the volume metering are presented in Table 1.

Table 1. Statistics of the volume metering results. The deviations between the patients(n=30) and within a patient sample are presented.

Results	Standard Deviation [µl]	Coefficient of Variation [%]
Between the Patients (n=30)	0,10	4,6
Within a Patient Sample	0,080	3,9

The coefficients of variation (CV) of the sample metering are low and there is only a slight difference in the CV between and within the patient samples. The CVs be even further reduced by re-design of the volume metering area. In the

current design, the cutting line of the sample plug and the pressure valve are relatively wide. Thus, the repeatability could be improved by designing a thinner cutting line and pressure valve.

Table 2 presents the results of the sample adhesion on channel walls ($n_{cartridge}$=5), cartridge base plate ($n_{cartridge}$=3) and cover plate ($n_{cartridge}$=4).

Table 2. Plasma adhesion on the cartridge. In each adhesion study one patient sample is used, and the number of cartridges used varies as mentioned above.

Adhesion on	Average [%]	Standard Deviation [%]	Coefficient of Variation [%]
Channel Walls	32.2	7.7	24.0
Cartridge Base	3.6	1.1	29.7
Cartridge Cover	4.6	0.7	14.8

The results indicate variation in the repeatability of the sample adhesion on the surfaces. It seems that the adhesion on the cover plate is more repeatable than on the injection moulded cartridge plate. The sample adhesion on the cartridge plates lowers the actual sample volume that takes part into the immunoreactions. In addition, due to the high relative error of the sample adhesion on the surfaces, variation in the sample volume increases compared to the metered sample volume. To reduce the sample adhesion and increase the repeatability, surface modifications or change of the cartridge material will be considered.

The average of the determined surface tension values of the plasma samples (n=5) in room temperature is 53.5 mN/m ± 1.4 mN/m with CV=2,6%. Each sample is measured three times. The results indicate good reliability. In the literature only the values of surface tension for whole blood, blood sediment and serum are found [6]. Compared to those, the results can be considered to be at an adequate level. Based on the results, there should not be a significant variation in the sample adhesion on the cartridge surfaces. Thereby, the causes of the variation in the sample adhesion on the channels and surfaces require more study.

The cross-sectional area of the volume metering area in the sample channel ($n_{cartridge}$=10) is 0.757 mm^2 ± 0.040 mm^2 with CV=5.23%. The estimated volume values in this work assume the depth of the channel to be constant. However, the cartridge dimensions do not affect the repeatability measurements presented in this paper. The relative error in the cross-section of the channel should be decreased to 1% – 2%. This indicates improvements to the injection moulding process or changing the manufacturing method. In the injection moulding, the higher tolerances might lead to increased costs. However, the repeatability of the cartridge dimensions is essential in immunoassays with strict requirements on the sample volume in relation to the reagent volume. Thereby, it is suggested that an electric injection moulding machine should be used because of the better overall repeatability.

CONCLUSION

The coefficients of variation in metering plasma sample volumes are 4.6% and 3.9% between the patients and within the patient samples, respectively, indicating that there are no significant differences between the patients compared to the variations within the samples from a patient. The coefficient of variation in the channel cross-sections fabricated using injection moulding is 5.2%. The sample adhesion studies indicate that sample adhesion is much stronger in the channels than on the base and cover plates.

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CONTACT

*P. Kallio, tel: +358-50- 0525546; pasi.kallio@tut.fi