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Research article

Feasibility study of revolutionizing animal healthcare with Lab-on-a-Chip technology: Case study on water buffalo blood analysis

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Abstract

Importance of the work: The development of the buffalo Lab-on-a-Chip (LOC) has the potential to revolutionize animal healthcare in developing countries by providing accessible and affordable diagnostics, particularly for assessing buffalo hematocrit levels.
Objectives: To develop an affordable and accessible LOC for buffalo health diagnostics, focusing on the evaluation of hematocrit levels.
Materials & Methods: Polydimethylsiloxane (PDMS) LOCs were fabricated using a stainless-steel mold. Buffalo blood samples, mixed with pure saline, were prepared for testing. Hematocrit levels were evaluated using manual capillary centrifugation. Sample flow patterns were observed to understand the behavior of the LOC.
Results: The functionality of the PDMS LOCs was validated using chicken blood samples, demonstrating their effectiveness. Notably, buffalo samples with low hematocrit values had sample flow toward the anode, while those with normal hematocrit values had flow toward the cathode.
Main finding: The buffalo LOC effectively distinguished between buffalo samples with low and normal hematocrit values, based on their flow behavior, showcasing its potential as an accessible diagnostic tool.

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Introduction

Lab-on-a-Chip (LOC) technology has the potential to improve the everyday lives of both humans and animals. LOCs are miniature in size (from a few millimeters to centimeters), portable and easily applicable in a variety of places, using a very small quantity of bodily fluid sample to provide the information of interest. Because of their efficiency and background based on the basic principles of microfluidics and simple electronics, LOCs could revolutionize conventional diagnostic methods and provide a less invasive approach, making bodily fluid analysis a much less traumatizing experience for the patient.

One of the most popular methods for manipulating bioparticles, including cell separation, sorting and trapping, is dielectrophoresis (DEP), which is defined as the interactions between dielectric particles and an electric field. Kwizera et al. (2021) presented a review study on novel techniques for producing a DEP force for microfluidic manipulation of bioparticles on a microscopic level, especially with human or animal cells, which is a very important field for further biotechnology and bioengineering research. According to them, various types of DEP-based (bio) microfluidic appliances could be classified based on how the non-uniformity is created using different types of microchannels, electrodes and media. In a review article, Emmerich et al. (2022) discussed microfluidic DEP devices as an effective tool for blood cell manipulation, based on variations in the electrophysiological characteristics of the cells. The collective spreading was studied of red blood counts (RBCs) from a human blood sample flowing in a microchannel by Chuang et al. (2018). They experimentally looked at how RBCs dispersed collectively in a straight microchannel and discovered that as shown in Fig. 1, the RBCs that were at first distributed on one wall of the microchannel relocated to the spanwise direction in downstream flow.

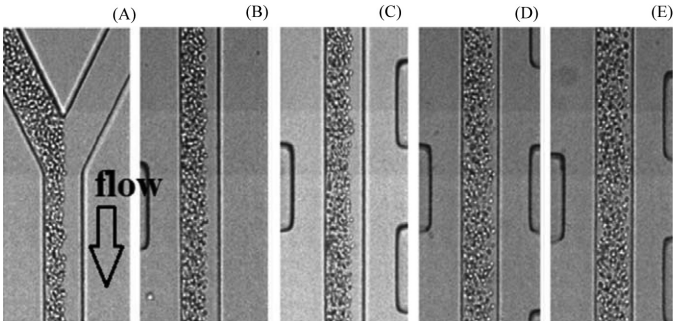

Fig. 1 Spreading of red blood cells along channel: original images at (A) confluence; (B) flow distance (x) = 1.0 mm; (C) x = 2.5 mm; (D) x = 5.0 mm; (E) x = 7.5 mm (sourced from Chuang et al., 2018)

Hematocrit (HCT) is a simple test to identify conditions, such as anemia or polycythemia, and also to monitor the response to treatment. The HCT decreases in anemia, where there are fewer RBCs in the circulating blood relative to the total volume of the blood. In contrast, in polycythemia, there is a higher number of RBCs in the blood, so the HCT increases. There have been few avian studies of the relationship between hematocrit and LOC conditions (Pramuanjaroenkij et al., 2013, 2018); however, this has not been the case with buffalos, perhaps because it has been simply assumed that buffalos in better physical condition or that are healthy have normal hematocrit levels. Hematocrit concentrations have been measured to investigate the health conditions of various animals. Manasawasde and Manasawasde (1986) experimentally investigated the mean corpuseular hemoglobin concentration (MCHC) of the infected fish, *Clarias batrachus* (Linnaeus), that was exposed to *Aeromonas hydrophila*, where the more *A. hydrophila*, the lower the MCHC. Later, Mahasawasde (1989) investigated the MCHC of the same fish species that had been exposed to sodium chloride solution for 3 d, with no significant differences in the MCHC with the different solution concentrations. Gongruttananun and Chotesangasa (2005) investigated the hematocrit values of re-hydrated and de-hydrated hens, where the greater the water deprivation, the greater the decrease in values. Gongruttananun and Guntapa (2012), investigated the hematocrit values of the hen, *Gallus domesticus*, using a red light treatment and found that the values tended to be lower than those for the other two treatments (natural daylight supplemented with fluorescent light and natural daylight supplemented with red light) in some periods of the study. Gongruttananun (2014) reported that the hematocrit value and eye morphology of the male birds measured at 16 weeks were influenced by light treatments. Later, Gongruttananun et al. (2017) continuously reported the hematocrit values of hens fed broken rice and cassava and reported significantly higher values than for those hens fed corn at the end of the molt period. Thongsarn et al. (2006) reported that the average hematocrits and hemoglobin concentrations of the Nile tilapia, *Oreochromis niloticus* were 25.80±3.71% and 7.05±1.08 g/dl, respectively. Prihirunkit et al. (2008) compared two methods (manual or automated) that were used to measure the hematocrit (Hct) and hemoglobin (Hb) concentrations of canines and felines. In the automated method, Hct could be calculated from the mean corpuscular red cell volume (MCV) and the red cell count (RBC), while the Hb concentration could be calculated by using pre-installed software designed

for veterinary applications. In the manual method, the Hct and Hb concentrations were evaluated based on capillary centrifugation and the cyanmethemoglobin (Hbcy) method, respectively. That work, in particular, reported in 2008 that both methods were accurate and precise; however, the automated method could not replace the manual method becaused the manual values could not be replaced by the automated values. Furthermore, Noosud et al. (2010) revealed the average Hct and Hb concentrations of Thai Bangkaew dogs were 36.38±0.77% and 13.10±0.32 g/dL, respectively.

Lu et al. (2018) published a paper in which they concluded that the viscosity of blood with low levels of hemoglobin would increase when the oxygen tension decreased; it may cease to flow without oxygen, despite its reduced hemoglobin concentration. Bento et al. (2018) examined the blood flow behavior of microchannel patterns on a microscopic level. The cell-free layer in an asymmetric microchannel design was examined in two different samples with varying hematocrit levels and the blood flow simulation pictures were analyzed using tools from the ImageJ image analysis software. For studying the flow of biological fluids, especially animal blood samples, Pramuanjaroenkij et al. (2018) presented the polydimethylsiloxane (PDMS) LOC made from two separate molds: a silicon wafer and a stainless mold. When pouring the PDMS into the LOCs cast from the stainless mold, nickel electrodes were used to insert the LOCs. It was found that the PDMS sheet easily adhered to a microscope slide and that PDMS could flow underneath the electrode without any issues.

In Thailand in the past, Thai swamp buffalos or water buffalos (lat. *Bubalus bubalis*) had a very important agricultural role, with Thai farmers using them as animal power for doing hard work, such as ploughing rice fields, planting, and harvesting rice. However, since the expansion of agricultural machinery technology, water buffalos are no longer used as a power source. Nowadays, Thai people conserve and breed them on farms and in animal healthcare facilities across the country, ensuring their good health. In other studies (Pramuanjaroenkij et al., 2013, 2018), the sample flow on LOCs was investigated using chicken blood. Certainly, avian erythrocytes differ from mammalian erythrocytes (Scanes, 2022). Nevertheless, LOCs could be locally fabricated and evaluated to determine their feasibility for use with water buffalo blood. Because there is a limited amount of research relating to LOCs for water buffalos, the focus of the current research was LOC blood analysis using blood samples taken from water buffalo specimens. The buffalo LOC with its collected information could be beneficial for everyday, rapid health check-ups on farms or at healthcare facilities. Keeping their buffalos healthy is a desired goal of every buffalo caretaker. While rapid health detection can provide less damage to the buffalos themselves, it can also prevent unwanted financial losses.

Materials and Methods

Lab-on-Chip preparation

The LOC architecture used in this research was based on research published by Pramuanjaroenkij et al. (2018). A silicon wafer mold and a stainless-steel mold were used to create the PDMS LOCs. The stainless-steel mold was properly cleaned before nickel electrodes were placed and the PDMS LOC cast was created.

Then, the PDMS and curing agent combination (5:0.5) was applied to the mold. The mold containing the PDMS mixture was placed on a hotplate at a constant temperature for 0.5 hr. After that, the mold was placed inside a vacuum chamber to remove cavitations inside the PDMS mixture. The mold was placed inside the oven for 3 hr and the temperature was controlled in the range 60–80°C. Then, the PDMS with the electrodes was removed from the mold and placed on a glass slide. The electrode connection was made by punching a hole in the PDMS on the top of the electrodes in the PDMS part after removal from the mold. Under a microscope (Fig. 2), the electrical connectors were manually inserted in the PDMS electrode chambers to generate the electric field, and the acrylic plates were sandwiched to connect each part of the LOC.

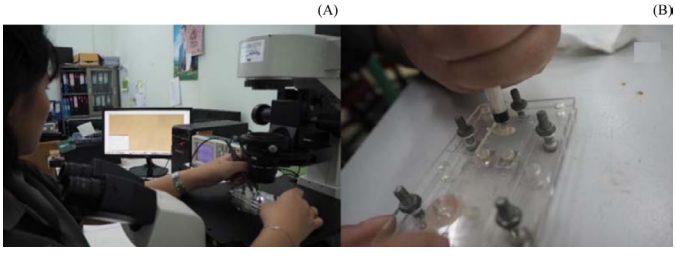

Fig. 2 Electrical connectors under microscope: (A) placing the Lab-on-a-Chip (LOC) on the microscope base to investigate sample-flow patterns; (B) injecting sample on LOC

Validation of chicken blood sample flow on Lab-on-a-Chip

Since the silicon-wafer-mold cast LOC was proven to be the most workable LOC reported in the literature (Pramuanjaroenkij et al., 2018), the PDMS LOC was first validated for its functionality by testing it with chicken blood. Then, it was tested with the buffalo blood samples taken from 10 chosen water buffalo specimens. The blood samples had been previously prepared by mixing each specimen with saline in a ratio of 0.5:1 to produce an adequate RBC solution for the experiment.

Traditional hematocrit test

The traditional hematocrit count started with professional caretakers by taking blood from the water buffalo in a glass tube. Next, the tube was centrifuged. After centrifugation, the blood component had separated into three distinct parts, with the bottom layer consisting of red blood cells (RBCs), the middle layer consisting of white blood cells (WBCs) and platelets, while the top layer contained plasma. This method of determining HCT using a Wintrobe hematocrit tube is known as the macro-hematocrit method (Fred, 2007). Then, the height of the RBC layer was divided by the total height to provide the hematocrit value. The hematocrit value is one of the parameters used to indicate animal health conditions. The reference interval of hematocrit values for water buffalo (*Bubalus bubalis*) heifers starts from 30.25%. In other words, if the water buffalo heifer has a hematocrit values outside the range 30.25–50.08%, their health conditions must be of concern (Ellah et al., 2014). The reference hematocrit value was 29.60% (Dhillon et al., 2010).

Water buffalo blood flow experiment in stainless-steel-mold Lab-on-a Chip

The water buffalo blood samples were examined using both the traditional hematocrit test in a standard laboratory and the LOC test. The results obtained from both analyses were correlated and jointly used to diagnose acceptable normal and abnormal health conditions.

Results

Lab-on-Chip preparation

At the beginning, the PDMS parts were cast on two molds: stainless-steel and silicon wafer (Pramuanjaroenkij et al., 2018), as shown in Fig. 3. The PDMS parts cast on the silicon wafer mold and the stainless-steel mold were placed on glass slides (Fig. 4) and spliced using two 5 mm acrylic sheets to construct the LOCs. The silicon-wafer-mold LOCs were tested by injecting saline inside the channels; some leakages and blockages (ceilings of the flow channels collapsed) were found. Fig. 5 shows the stainless-steel-mold LOCs that were prepared for the sandwiching process. After the sandwiching process, a few LOCs prepared from the stainless-steel mold could not be used because the glass slides were broken during tightening of the screws on the acrylic sheets. Greater care was taken when attaching the acrylic sheets to the LOC test set, with the six screws being tightened gently to avoid breaking the glass slides. Then, saline was injected into the LOC devices prepared from the stainless-steel mold. No leakage or blockages were found; these newly layered LOCs were workable. The LOCs prepared from the stainless-steel mold were chosen for the next step, with the steps to fabricate these LOCs prepared repeatedly displayed in Fig. 6.

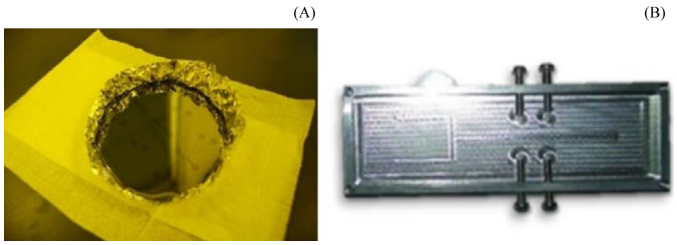

Fig. 3 Molds: (A) silicon wafer; (B) stainless-steel

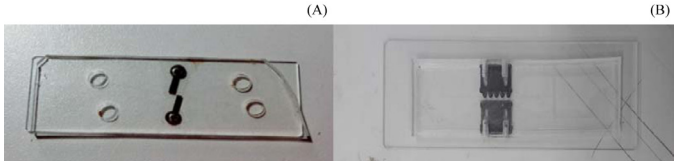

Fig. 4 LOCs cast on the silicon wafer mold (A); and the stainless-steel mold (B)

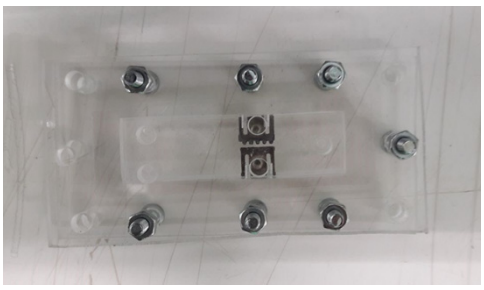

Fig. 5 Lab-on-a-Chip sandwiched by pair of s acrylic sheets formed in stainless-steel mold

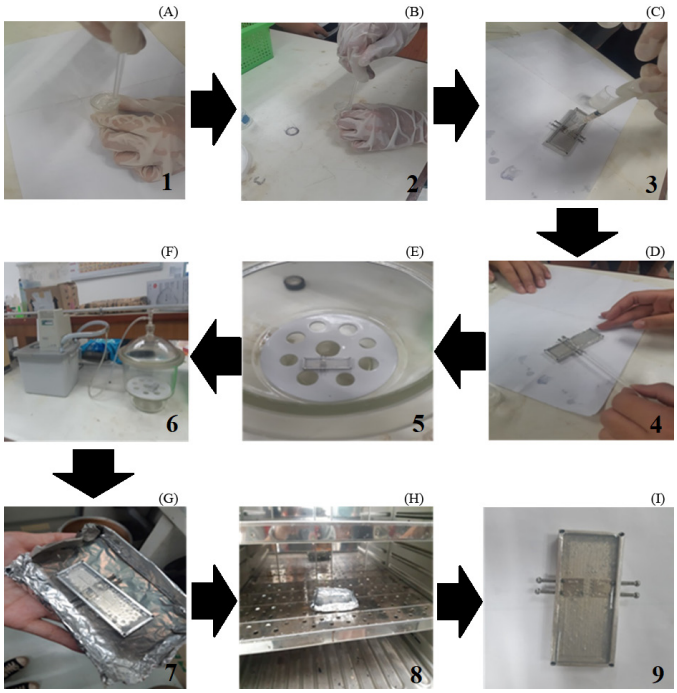

Fig. 6 Steps in Lab-on-a-Chip fabrication process: (A)–(B) mixing water with polydimethylsiloxane (PDMS) to make homogeneous mixture (requiring about 5 min); (C) pouring homogeneous mixture into mold; (D) inserting electrodes in mold; (E)–(F) placing mold inside vacuum chamber for 30 min; (G)–(H) placing mold with mixture into oven; (I) completed PDMS sandwiched between glass slides

Validation of chicken blood sample flow on LOC

The LOCs cast from the stainless-steel mold were tested with biological samples prepared from the blood of healthy and unhealthy chickens. The chicken health conditions were checked by chicken professional caretakers based on the physical appearance and activity of the poultry. It was found that the biological samples flowed differently, with the flow velocity of the healthy chicken blood being faster than for the unhealthy chicken blood. In addition, the unhealthy chicken blood clotted the channels faster than the healthy chicken blood. The photographs of the sample blood flow taken during the LOC tests were analyzed using the ImageJ™ image-analysis software. The results obtained from both the traditional hematocrit test and the ImageJ analysis were compared to identify the correlations leading to the correct diagnosis of the normal and poor health conditions. Figs. 7 and 8 display the different RBC profiles in both the microscopic and ImageJ images. In Fig. 8, there is an evenly distributed RBC profile in both images, while in Fig. 7, the RBC profile has some gaps and uneven spacing in both images. The stainless-steel-mold LOCs were validated using the chicken blood flow results because validation was suitable with the previous chicken blood investigation on the LOCs (Pramuanjaroenkij et al., 2018).

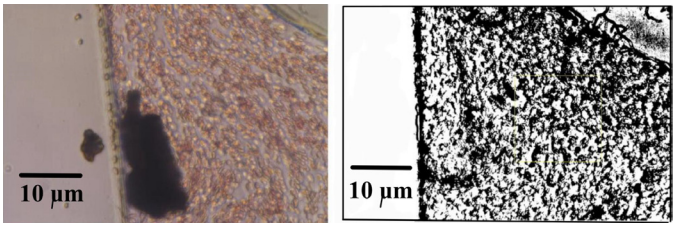

Fig. 7 Microscopic and ImageJ images of unhealthy chicken blood

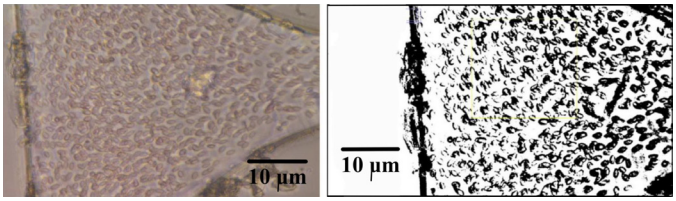

Fig. 8 Microscopic and ImageJ images of healthy chicken blood

Traditional hematocrit test

After the buffalo specimens had been prepared, as shown in Fig. 9A, the traditional standard hematocrit test was performed at the Department of Animal Science, Kasetsart University, Chalermphrakiat Sakon Nakhon Province campus, Thailand in parallel to analyzing the blood samples on the LOCs. The experimental investigation refers to the limit of quantification (LOQ), which is the smallest concentration of an analyte in a test sample with acceptable repeatability and accuracy (Vashist and Luong, 2018). The comparison of the results with those obtained on LOCs required determining the hematocrit levels of each blood sample. Fig. 9 shows the procedure for the traditional hematocrit test, which was carried out in accordance with the prescribed protocol (Thongsarn et al., 2006; Prihirunkit et al., 2008; Noosud et al., 2010). The hematocrit results obtained from all 10 buffalos are reported in Table 1, with five of the animals having acceptably normal health, while the other five had low hematocrit values (lower than 30), which indicated an abnormal health condition. These hematocrit values were used to indicate the health conditions. The buffalo blood samples were mixed with pure saline to determine physical appearance. Fig. 10 presents a sample with a hematocrit value higher than 30, while Fig. 11 presents a

sample with a hematocrit value lower than 30, As can be seen from Figs. 10 and 11, the physical appearance of the samples in both figures appeared the same to the naked eye. The traditional hematocrit evaluation takes time, due to sample preparation and using centrifugation, which is available only in laboratories. In contrast, sample investigation using the LOCs used less sample and produced sample flow patterns quicker than the traditional evaluation method.

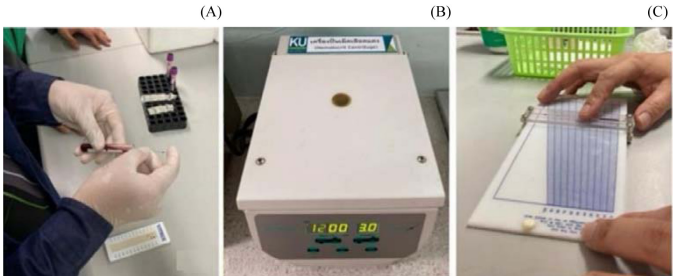

Fig. 9 Traditional hematocrit test: (A) placing sample inside glass tube; (B) centrifuging glass tube to separate into three sample layers; (C) height measurement of red blood count layer

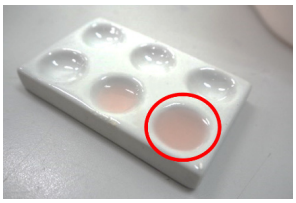

Fig. 10 Visual characteristics of buffalo blood with normal hematocrit level mixed with pure saline

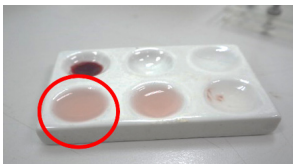

Fig. 11 Visual characteristics of buffalo blood with low hematocrit mixed with pure saline

Water buffalo blood flow experiment in stainless-steel-mold Lab-on-a-Chip

The water buffalo's health conditions were hard to distinguish based on physical appearance and activity. Therefore, the biological sample from each buffalo was separated into two parts. The first part was investigated in the laboratory with the hematocrit values determined using traditional hematocrit counting, while the other part was investigated using the LOCs.

For the LOC test, the buffalo blood part was mixed with pure saline and then an electric field was produced on the LOC using 5 V and 50 Hz alternating currents. The sample stream inside the LOCs was observed and recorded as it flowed smoothly and continuously. Since the microscope's field of view could not cover the complete LOC clearly, the LOC flow is shown schematically in Fig. 12. Noticeably, one set of buffalo blood samples flowed and moved toward the negative electrode (cathode), as shown in Fig. 12 (A). A part on Fig. 12 was enlarged and is shown in Fig. 13. Another sample set flowed and moved toward the positive electrode (anode), as shown by the schematic diagrams in Fig. 12 (B). Both schematic patterns are also noted in Table 1.

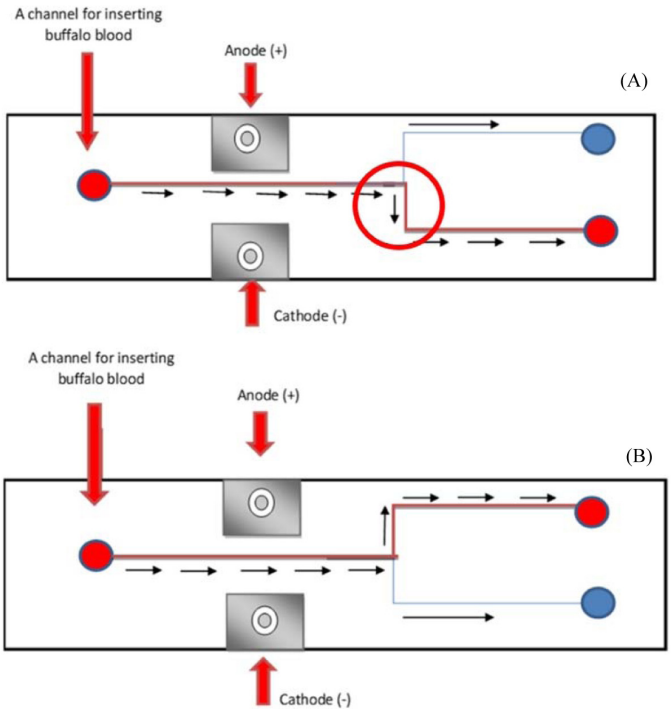

Fig. 12 Schematics of Lab-on-a-Chips displaying blood flow: (A) toward cathode; (B) toward anode

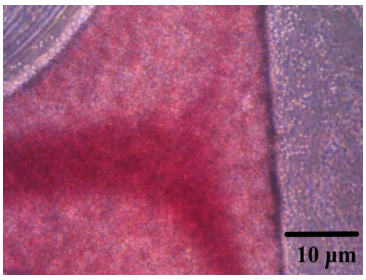

Fig. 13 Image for part of field of view on microscope for Lab-on-a-Chip displaying blood flow toward cathode

The correlation between the hematocrit values and sample flow patterns on the LOCs are described in Table 1. Notably, for the buffalo samples with low hematocrit values, the sample flow on the LOC moved toward the anode, while for the buffalo samples with normal hematocrit values, the sample flow on the LOC moved toward the cathode.

Discussion

One main advantage of the LOC application is the very small amount of animal blood used because the blood sample is diluted in normal saline before being dropped onto the LOCs. The sample amount and test period required by the LOC application were less than for the traditional hematocrit test. Using a small amount of blood should mean less discomfort in drawing the sample and a shorter, less stressful situation for the animals, which was further facilitated by the blood samples being taken by professional animal caretakers. The limitations of this work consisted of only a single buffalo species was sampled, health condition were no a part of the sampling procedure and the number of buffaloes sampled was low (10). The buffalo species and the number of animals were selected by the Department of Animal Science, Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus. The buffalo's health conditions were not intentionally sampled. In the current work, the devices were prepared for testing as instructed in the literature (Pramuanjaroenkij et al., 2018). The testing of each blood sample was repeated three times on the stainless-steel-mold LOCs. According to the traditional hematocrit results, the hematocrit values may differ among a herd of buffaloes. The lowest and highest hematocrit values were 25 and 36.5, respectively. Noticeably, the clear parts of the buffalo blood samples flowed in both directions of the LOC channels when the flow was observed with the naked eye under a microscope, while the RBCs flowed primarily in one direction, according to the results in Table 1.

Table 1 Comparison between hematocrit measurements and Lab-on-a-Chip blood flow analysis

Type	Number of repetitions	Hematocrit		Blood flow		Status
		Value (%)	State	Direction	Polarity	
1. Water buffalo 1; code 2c014	1	30	Low	Upward (↑)	Plus (+)	Abnormal
	2	30	Low	Upward (↑)	Plus (+)	Abnormal
	3	30	Low	Upward (↑)	Plus (+)	Abnormal
2. Water buffalo 2; code 2c026	1	27.5	Low	Upward (↑)	Plus (+)	Abnormal
	2	27.5	Low	Upward (↑)	Plus (+)	Abnormal
	3	27.5	Low	Upward (↑)	Plus (+)	Abnormal
3. Water buffalo 3; code 2c045	1	27.5	Low	Upward (↑)	Plus (+)	Abnormal
	2	27.5	Low	Upward (↑)	Plus (+)	Abnormal
	3	27.5	Low	Upward (↑)	Plus (+)	Abnormal
4. Water buffalo 4; code 2c051	1	27.5	Low	Upward (↑)	Plus (+)	Abnormal
	2	27.5	Low	Upward (↑)	Plus (+)	Abnormal
	3	27.5	Low	Upward (↑)	Plus (+)	Abnormal
5. Water buffalo 5; code 2c047	1	25	Low	Upward (↑)	Plus (+)	Abnormal
	2	25	Low	Upward (↑)	Plus (+)	Abnormal
	3	25	Low	Upward (↑)	Plus (+)	Abnormal
6. Water buffalo 6; code 2c030	1	32.5	Normal	Downward (↓)	Minus (-)	Normal
	2	32.5	Normal	Downward (↓)	Minus (-)	Normal
	3	32.5	Normal	Downward (↓)	Minus (-)	Normal
7. Water buffalo 7; code 2c056	1	30	Normal	Downward (↓)	Minus (-)	Normal
	2	30	Normal	Downward (↓)	Minus (-)	Normal
	3	30	Normal	Downward (↓)	Minus (-)	Normal
8. Water buffalo 8; code 2c033	1	35	Normal	Downward (↓)	Minus (-)	Normal
	2	35	Normal	Downward (↓)	Minus (-)	Normal
	3	35	Normal	Downward (↓)	Minus (-)	Normal
9. Water buffalo 9; code 2c041	1	35	Normal	Downward (↓)	Minus (-)	Normal
	2	35	Normal	Downward (↓)	Minus (-)	Normal
	3	35	Normal	Downward (↓)	Minus (-)	Normal
10. Water buffalo 10; code 2c046	1	36.25	Normal	Downward (↓)	Minus (-)	Normal
	2	36.25	Normal	Downward (↓)	Minus (-)	Normal
	3	36.25	Normal	Downward (↓)	Minus (-)	Normal

State refers to levels of hematocrit.
Status refers to buffalo's health condition.

The samples with low hematocrit values only flowed upward to the anode, while the samples obtained from the normal-health-condition buffaloes flowed downward to the cathode. This initial research on testing the buffalo blood using the LOCs showed the potential of the LOCs to be used with buffalo blood and with the blood of other animals, as well as suggesting the potential for exploring more opportunities for stainless-steel-mold LOC applications. Further studies could be performed on measuring the physical and electrical properties of the flow of the samples in the LOC channels and to the anode and cathode of the LOCs. In addition, correlations could be investigated between the properties and animal health conditions.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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