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Research Article

Keywords: cell tracking velocimetry, hematology analyzer, blood testing, point of care, anemia screening, red blood cells

Posted Date: May 20th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-526087/v1

Version of Record: A version of this preprint was published at Scientific Reports on February 1st, 2022.
See the published version at https://doi.org/10.1038/s41598-022-05654-5.

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ABSTRACT

Anemia and iron deficiency continue to be the most prevalent nutritional disorders in the world, affecting billions of people in both developed and developing countries. The initial diagnosis of anemia is typically based on several markers, including red blood cell (RBC) count, hematocrit and total hemoglobin. Using modern hematology analyzers, erythrocyte parameters such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), etc. are also being used. However, most of these commercially available analyzers pose several disadvantages: they are expensive instruments that require significant bench space and are heavy enough to limit their use to a specific lab and leading to a delay in results, making them less practical as a point-of-care instrument that can be used for swift clinical evaluation. Thus, there is a need for a portable and economical hematology analyzer that can be used at the point of need. In this work, we evaluated the performance of a system referred to as the cell tracking velocimetry (CTV) to measure several hematological parameters from fresh human blood obtained from healthy donors. Our system, based on the paramagnetic behavior that methemoglobin containing RBCs experience when suspended in water after applying a magnetic field, uses a combination of magnets and microfluidics and has the ability to track the movement of thousands of red cells in a short period of time. This allows us to measure not only traditional RBC indices but also novel parameters that are only available for analyzers that assess erythrocytes on a cell by cell basis. As such, we report, for the first time, the use of our CTV as a hematology analyzer that is able to measure red cell volume or MCV, red cell hemoglobin mass or MCH, hemoglobin concentration (MCHC), red cell distribution width (RDW) and the percentage of hypochromic cells, which is an indicator of insufficient marrow iron supply that reflects recent iron reduction. Our initial results indicate that most of the parameters measured with CTV are within the normal range for healthy adults. Only the parameters related to the red cell volume (primarily MCV and RDW) were outside the normal range. We observed significant discrepancies between the MCV measured by our technology (and also by an automated cell counter) and the manual MCV measured through the hematocrit obtained by packed cell volume method, which are attributed to the artifacts of plasma trapping and cell shrinkage. While there may be limitations for measuring MCV, this device offers a novel point of care instrument to provide rapid RBC parameters such as iron stores that are otherwise not rapidly available to the clinician. Thus, our CTV is a promising technology with the potential to be employed as an accurate, economical, portable and fast hematology analyzer after applying instrument-specific reference ranges or correction factors.

Keywords: cell tracking velocimetry, hematology analyzer, blood testing, point of care, anemia screening, red blood cells

INTRODUCTION

Despite global progress achieved in medicine and science, anemia continues to be one of the most prevalent disorders. Anemia is defined by the World Health Organization (WHO) as a condition in which the hemoglobin (Hb) concentration in blood is lower than 130 g/L in men and lower than 110 g/L in pregnant women [1]. It affects more than 30% of the world's population, of which more than 50% suffers from iron deficiency anemia (IDA) [2, 3]. In fact, iron deficiency (ID) is the most widespread nutritional disorder in the world, afflicting between 2 and 4 billion people worldwide [4, 5]. IDA is particularly prevalent for children and women in both developing and developed countries [6-9]. ID leads to the suppression of Hb synthesis, induces metabolic disorders, affects cognitive and motor development, and causes fatigue and decreased productivity. One of the most common causes to develop iron deficiency is blood loss; for example, iron deficiency anemia may be developed after blood donation if iron stores are limited [6]. In fact, 35% of the 9 million blood donors in the United States is estimated to be iron deficient [10].

ID/IDA can be diagnosed by different biochemical analysis. Determination of red blood cell (RBC) count, hematocrit (Hct) and Hb are routine laboratory test to determine the presence of anemia. Based on the work by Wintrobe in the early 1930s, RBC indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) or mean corpuscular hemoglobin (MCH) were established to characterize the RBCs of anemic patients [11]. These are valuable data for the diagnosis of ID or IDA and can be easily acquired by automated blood cell counters. In fact, modern analyzers such as ADVIA (Siemens Healthineers Inc.) uses a range of technologies such as flow cytometry, chemical reaction and spectral absorption reading to provide several indexes, such as reticulocyte count, (early index of ID, as reticulocytes exist in the circulation for only 1-2 days), hypochromic RBC count, (cells with a Hb concentration lower than 28 g/dL, an indicator of insufficient marrow iron supply that reflects recent iron reduction), etc. [6, 11, 12]

Although these instruments are accurate, reproducible and sometimes fast, the cost of the newest, high-volume analyzers is high and can vary from a single instrument of approximately $75,000 to an automated multiple-instrument system in excess of $200,000 dollars [13]. Also, these are large and heavy equipment, sacrificing portability; thus, samples collected off-site present a time delay in analyzing the sample, introducing risks of inaccuracy and contamination. Furthermore, they may require trained laboratory technicians, high blood volume requirements, and costly reagents for sample pretreatment [14]. Moreover, the automated hematology analyzers are not standardized among manufacturers due to patent issues, which makes necessary the establishment of instrument-specific reference ranges and clinical decision values [15, 16].

We have previously developed a system referred to as the cell tracking velocimetry (CTV), to measure the mass of Hb in fresh RBCs on a cell by cell basis based on the pioneering work of Pauling and Coryell's studying the magnetism of Hb at different chemical states [17, 18]. CTV uses a combination of microscope camera and magnetic, microfluidic channel within a well-defined magnetic energy gradient to track the movement of cells and particles under the direct influence of magnetic and gravitational fields. Mathematically, the magnetically and gravitationally induced velocities, u_m and u_s, can be described as follows:

$$u_m = \frac{(\chi_{Cell} - \chi_{Fluid})V_{Cell}}{3\pi D_{Cell}\eta} S_m \quad (1)$$

$$u_s = \frac{(\rho_{Cell} - \rho_{Fluid})V_{Cell}}{3\pi D_{Cell}\eta} g \quad (2)$$

where the subscripts cell and fluid refer to the cell and suspending fluid, **χ** is the magnetic susceptibility, ρ is the density, D and V are the diameter and volume of a cell (particle), η is the viscosity of the suspending fluid and g is the acceleration due to gravity (9.8 m/s^2). S_m, the magnetic energy gradients, is defined by:

$$S_m = \frac{|\nabla B^2|}{2\mu_0} \quad (3)$$

where μ_0 and B are the permeability of free space and the magnetic flux density at the source.

Rearranging equation (2) can yield the MCV, taking into account the disc shaped fresh RBC, as follows:

$$D_{RBC} = \left[\frac{1.349 * u_s * \eta * 18}{\Delta\rho * g}\right]^{0.5} \quad (4)$$

$$MCV_{CTV} = h_{RBC} * \pi * \left[\frac{D_{RBC}}{2}\right]^2 \quad (5)$$

where 1.349 is the sedimentation rate (u_s) drag factor found by Zhbanov *et al*. [19] and the hRBC is the average RBC thickness (2.25 µm). From the MCV distribution, the RBC distribution width (RDW_{CTV}) can also be obtained.

Previously, the relationship between the magnetic and settling velocity and the amount of hemoglobin in the RBC, accounting for volume, has been described in detail [20, 21]:

$$MCHC_{CTV} = \frac{\left(\frac{u_m}{u_s}\right)(\Delta\rho)\left(\frac{g}{S_m}\right)}{(\chi_{m,metHb} + \chi_{m,globin} - \chi_{H_2O}) * V_{m,Hb}} * MW_{Hb} \quad (6)$$

$$MCH_{CTV} = \frac{9 * 2^{0.5}\pi}{S_m * (\chi_{m,metHb} + \chi_{m,globin} - \chi_{H_2O}) * V_{m,Hb}} * \left[\frac{(1.23 * u_m) * (1.23 * u_s)^{0.5} * \eta^{1.5}}{\Delta\rho^{0.5} * g^{0.5}}\right] * 10^3 * MW_{Hb} \quad (7)$$

where $V_{m,Hb}$ = 48.23 L/mol is the molar volume of metHb, $\chi_{m,globin}$ = -37,830 × 10^{-9} L/mol is the molar susceptibility of the globin chain, and χ_{H2O} = -12.97 × 10^{-9} L/mol is the molar susceptibility of water. The molar susceptibility of the deoxyHb heme group is $\chi_{m,deoxyHb}$ = 50,890 × 10^{-9} L/mol, and that of metHb heme group is $\chi_{m,metHb}$ = 56,000 × 10^{-9} L/mol (all in CGS system of units). From these data, the percentage of RBCs with abnormal Hb concentration, such as hypochromic RBCs, which is a useful parameter for the detection of anemias, can be easily calculated ($Hypo_{CTV}$).

Using these features, the CTV has the potential to be employed as a portable, low-cost hematology analyzer. In this work, we report for the first time the use of our CTV as a hematology analyzer able to measure both traditional RBC indices (MCV, MCHC and MCH) and novel indices such us the percentage of hypochromic RBCs or RDW, showing for the first time the capability of this technology to perform high precision flow cytometry analysis.

MATERIALS AND METHODS

Sample preparation

Thirty-one whole blood samples from healthy donors were collected following informed consent according to a protocol approved by the Institutional Review Board (IRB) of The Ohio State University (protocol number 38334). All experiments were performed in accordance with relevant guidelines and regulations. A total of approximately 8 mL of whole blood was drawn into a 10 mL collection tube containing EDTA anticoagulant. The samples were separated into two aliquots, each designated for whole blood analysis and RBC analysis. The RBC aliquots were further processed and washed in PBS to separate the RBCs from plasma (three times with 200 x g centrifugation for 5 minutes).

Coulter Counter analysis

All samples were introduced to our automated cell counter, B23005 Multisizer 4e Coulter Counter (CC, Beckman Coulter, CA), to measure the cell concentration as well as volume, MCV. The indicators measured with this instrument are denoted by a CC subscript (i.e. RBC $count_{CC}$, MCV_{CC}).

Spectrophotometric analysis

Total hemoglobin, Hb, is measured from whole blood samples by using the spectrophotometric method commonly employed in clinical laboratories, as described in our previous work [21]. Briefly, the samples obtained for spectrophotometric Hb concentration determination were diluted (4X) in deionized water in order to lyse the RBCs. The samples were allowed to sit for 30 minutes at 6^{o}C to ensure complete lysis. Then, the lysed cells were pelleted via tabletop centrifugation (2000 x g). The concentration of Hb in the supernatant was assessed via OLIS Spectral-works (Olis, Inc., GA), where the diluted samples were further diluted to ensure that the absorbance around the Q bands (~540nm and 575nm) was between 0.1 and 1.0 for accuracy in readings according to Beer-Lambert's law. Each sample was tested in triplicate and the mean and standard deviation of the absorbance were calculated. The Hb measured by this method is denoted by a spec subscript (Hb_{spec}).

CTV analysis

The calibration and operating procedure for using CTV to magnetically characterize RBCs has been described in previous reports [21, 22]. Supplementary **Figures S1 and S2** report the working principle and the optimization of the CTV operation procedure. Briefly, RBCs were oxidized with a sodium nitrite ($NaNO_2$) solution to turn them into methemoglobin containing RBCs (metHb-RBCs), then the metHb-RBCs were introduced into the CTV instrument at a concentration of 1 million cells/mL and the channels were sealed on both ends using Hamilton 1-1 valves (Hamilton Company, NV) to dampen any flow disturbances. After approximately 20 seconds, images of cell movement were captured (50 images at 1 s interval) and the captured images were further processed using an in-house analysis program that can convert the captured movement of the cells into magnetic and settling velocity of the cells.

Packed-Cell Volume

The conventional Hct test, also known as a packed-cell volume (PCV) test, was performed by the micro-hematocrit centrifugation method. A hematocrit capillary tube (Drummond Scientific Company) was filled with whole blood and capped on one end with a clay sealant. Then, the tubes were centrifuged at 17,000 x g for 5 minutes in a Sorvall Legend Micro 17 (Thermo Scientific), and the Hct was read from the

capillary tube once aligned with a provided chart and the result was round to the nearest half percent. The Hct measured by this method is denoted by a PVC subscript (Hct_{PVC}).

Other estimations for the erythrocyte indices

As stated before, the RBC indices are useful to determine if a patient is anemic, as well as to initially classify the anemia state. Hb, Hct and RBC count are usually employed to estimate three indices: MCV, MCHC and MCH. While these indices can be directly measured on CTV or on automated cell counters, here, we are also going to estimate them from the Hb, RBC count and Hct values, for comparison purposes. Thus, the average MCV, defined as the average volume of RBCs, and usually expressed in femtoliters (fL), can be obtained from the Hct and the RBC count, as follows:

$$\mathrm{MCV_{ave}} = \frac{\mathrm{Hct\ (L/L)}}{\mathrm{RBC\ count\ (x\ 10^{12}/L)}} \tag{8}$$

On the other hand, the MCHC is the average concentration of hemoglobin in the RBCs, usually expressed in g/dL, and is calculated from the Hb and Hct as follows:

$$\mathrm{MCHC_{ave}} = \frac{\mathrm{Hb\ (g/dL)}}{\mathrm{Hct\ (L/L)}} \tag{9}$$

Finally, the MCH measures the average weight of hemoglobin in individual RBCs, usually expressed in pg, and is calculated from the Hb and RBC count as follows:

$$\mathrm{MCH_{ave}} = \frac{\mathrm{Hb\ (g/dL)}}{\mathrm{RBC\ count\ (x\ 10^{12}/L)}} \tag{10}$$

These three estimated indices (ave subscripts) will be calculated in this work based on the Hct, Hb and RBC count provided by the traditional methods (Hct_{PVC}, Hb_{spec} and RBC $count_{CC}$) and compared with direct measurements taken from CTV and Coulter Counter.

RESULTS AND DISCUSSION

A total of 31 RBC samples were collected, and the following parameters were measured: Overall Hb concentration, Hct, RBC count, MCV, RDW, MCH, MCHC and Hypo, by using a combination of different methods as explained above. The results of the measurements are presented in **Table 1**. As expected, most of the test results show a healthy blood status of the donors, as most of the parameters are within the normal range [23]. However, some parameters related to the RBC volume (MCV, MCHC and RDW) and measured by CC or CTV are outside the normal range. These discrepancies are thoroughly analyzed and discussed in the following.

Table 1. Hb concentration, Hct, RBC count, MCV, RDW, MCH, MCHC and Hypo measured by different methods for 31 fresh human blood samples.

Donor #	Gender	Age	Hb_{spec} (g/dL)	Hct_{PVC} (%)	RBC $count_{CC}$ (10^6 cells/µL)	MCV_{ave} (fL)	MCV_{CC} (fL)	RDW_{CC} (%)	MCV_{CTV} (fL)	RDW_{CTV} (%)	MCH_{CTV} (pgHb)	$MCHC_{CTV}$ (g/dL)	$Hypo_{CTV}$ (%)
1A	M		17.76	51	6.60	76.80	49.76	26	52.88	20	36.11	50.59	9
1B	M		7.05	48	5.94	79.59	49.2	30	47.62	22	26.19	44.31	9
1C	M	29	15.34	46	6.30	71.93	47.46	28	52.15	23	29.37	44.03	8
1D	M		17.70	51	4.97	103.20	47.65	30	47.65	24	25.46	43.13	13
1E	M		17.18	49	5.52	89.03	50.65	24	49.77	30	35.85	61.07	3
2A	F		15.15	46	5.70	80.80	54.97	26	51.68	22	38.48	58.89	10
2B	F		16.68	46	4.60	100.25	56.95	28	52.49	19	28.24	40.13	10
2C	F		15.78	45	5.59	80.55	56.56	24	49.95	26	29.34	48.98	15
2D	F		15.78	45	4.36	103.10	56.07	24	51.17	23	31.03	49.39	6
2E	F	31	14.92	45	4.36	70.46	53.53	23	50.40	24	26.64	46.13	5
2F	F		15.62	45	4.36	10.32	55.69	23	54.28	35	34.49	52.28	10
2G	F		15.62	45	4.38	102.65	57.08	22	52.64	34	37.43	61.75	26
2H	F		15.14	46	4.38	104.93	55.24	23	52.01	21	25.75	39.12	16
2I	F		17.28	48	4.38	109.49	55.51	22	51.15	23	34.10	51.14	5
3A	M		17.22	48	5.20	91.56	52.67	25	52.33	23	31.99	48.45	29
3B	M		18.77	50	5.65	90.32	53.74	25	47.86	20	27.96	46.35	5
3C	M	32	16.02	48	5.07	94.74	50.67	24	50.17	21	25.71	38.24	23
3D	M		17.47	50	4.40	110.88	55.17	22	49.92	22	30.52	49.85	5
3E	M		16.37	50	4.40	80.57	50.67	28	47.89	22	26.10	45.54	7
4A	F	24	15.26	44	5.30	83.03	55.32	24	50.43	23	30.62	47.37	5
4B	F		15.45	44	5.32	82.67	52.27	27	45.62	27	24.25	46.09	12
5A	M		15.26	43	4.85	89.60	52.66	27	50.52	26	27.56	43.01	12
5B	M		15.57	45	4.85	102.74	53.49	22	48.94	25	30.82	50.31	12
5C	M	60	15.98	45	4.85	98.11	52.68	22	50.28	21	43.75	66.04	7
5D	M		15.98	44	4.37	99.87	53.94	20	48.36	24	30.83	50.28	10
5E	M		15.13	45	4.37	94.90	53.87	25	52.02	22	23.04	33.27	29
6A	F		12.76	42	4.85	75.05	43.4	27	43.40	25	15.40	28.92	55
6B	F		12.90	43	4.40	86.77	44.56	24	45.60	19	41.67	74.10	3
6C	F	22	12.90	50	4.70	89.13	45.42	23	43.84	29	20.70	40.12	34
6D	F		12.96	42	4.38	86.24	44.64	24	52.18	19	24.38	35.60	22
6E	F		12.73	42	4.38	92.78	44.61	31	41.57	24	24.51	50.06	5
Average			**15.35**	**46**	**4.92**	**88.13**	**51.81**	**25**	**49.57**	**24**	**29.62**	**47.89**	**14**

Mean Corpuscular Volume

The estimation of MCV (Eq. 8) calculated from the Hct obtained by the PCV method and the RBC count obtained from CC, gives an MCV range of 70-110 fL, which matches well with the literature values reported for healthy individuals [23]. However, measurements from the Coulter Counter and CTV, using equation (5), give us values around 50 fL. The close overlap of size distribution data from the CC and the

CTV is shown in **Figure 1**. It could be seen from the bar graph that MCV_{ave} is consistently different from MCV_{CC} and MCV_{CTV}.

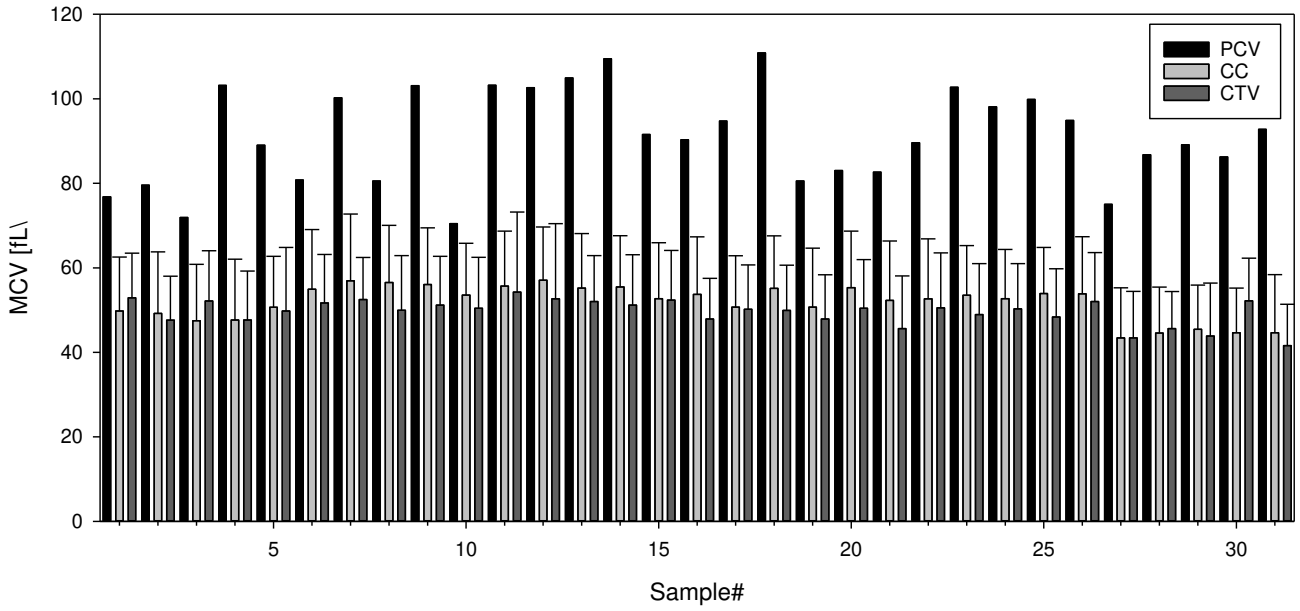

Figure 1. Comparison between the MCV_{CC}, MCV_{CTV} and the MCV_{ave} value calculated from the Hct obtained by the PCV method for the total 31 samples.

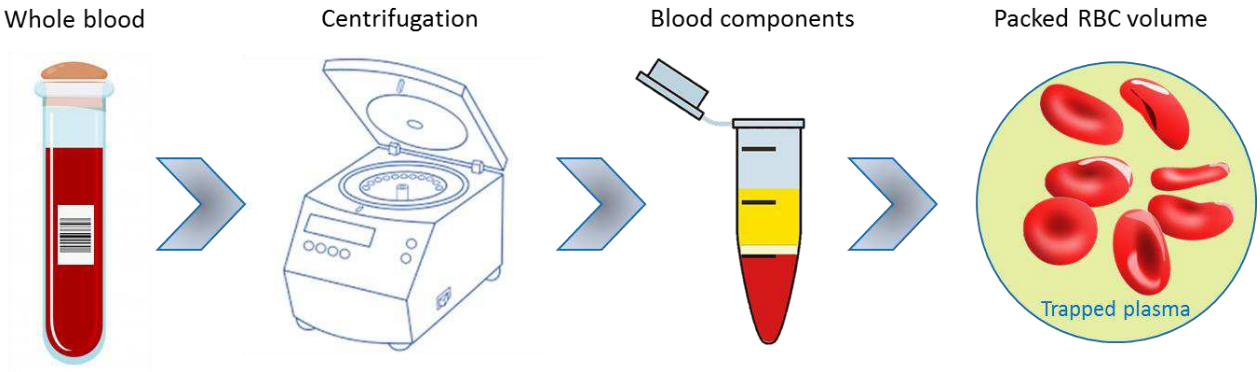

Figure 2. Schematic representation of plasma entrapment during Hct measurements by the PCV method.

As mentioned, for the determination of MCV_{ave} (Eq. 8), data obtained from PCV and CC are employed. Values from CC related to RBC count are within the normal range of healthy patients, as seen in Table 1 [23]. Values of Hct obtained from PVC are also within the normal range (around 45%). The disagreement between the MCV obtained by Eq. 8 and the value obtained from CC and CTV may be due, in part, to the fact that the PCV method uses centrifugal force to pack the RBCs in a hematocrit capillary tube, which inevitable suffers from plasma entrapment (see **Figure 2**). Thus, the discrepancy in MCV between the different methods could be in part attributed to the plasma entrapment when measuring Hct with this

method. Nevertheless, it has been reported that less than 5% of plasma is trapped in the packed RBC layer during centrifugation [23]. For example, Paterakis *et al*. [24] have suggested that the trapped plasma constitutes less than 3% for any normal or abnormal samples, including oxygenated sickle cells.

Another plausible explanation for this disagreement is the fact that RBCs are deformable and some techniques might not take into account the erythrocyte deformability; this could lead to an underestimation of the MCV measured on CC or CTV, as has been previously reported by others. For example, d'Onofrio *et al*. [25] stated that electronic measures of MCV could be underestimated. In fact, these authors claimed that electronic Hct (measured on CC by using RBC count and MCV) is a redundant parameter that could be abandoned, and that there are significant discrepancies between the manual Hct (obtained by PCV method), with the artifacts of plasma trapping and cell shrinkage, and the automated measurements. Thus, they suggest that calculation of Hct from the mean or the sum of pulse sizes and RBC count leads to systematic underestimation of automated Hct (and MCV) compared to manual. Finally, Brugnara *et al*. [26] reported that individual MCV measures on different hematology analyzers is dependent on the technology used and that impedance-based instruments (CC) might underestimate MCV in hypochromic RBCs. The differences on the technologies employed, along with the reporting of such impedance-based instruments might not account for the deformability of RBCs [27], could contribute to the disagreement on the MCV obtained with the different techniques. So far, no internationally accepted reference method has been published for measuring MCV [28].

Due to the previous explanations, and most importantly, since there is no reference method to measure MCV, we can conclude that the CTV could potentially be employed for measuring MCV along with other hematological parameters as long as reference values or correction factors are established. Even though RBC deformability might not be considered on CTV, the values obtained from both CTV and Coulter Counter are very similar, and Coulter Counter is already considered as an accurate instrument for measuring cell and particle volume distributions.

The Coulter Counter calculates the volume of the sample by measuring the voltage difference in electrolytes between the inside/outside the gating tube every time a sample passes through the orifice of the tube, and further process this value into volume. The red cell volume measured by the Coulter Counter is also very close to the value reported by the CTV, which uses cell sedimentation velocities to calculate their volume. For example, **Figure 3** reports the volume distributions of one RBC sample measured on both CC and CTV, showing nearly identical distributions.

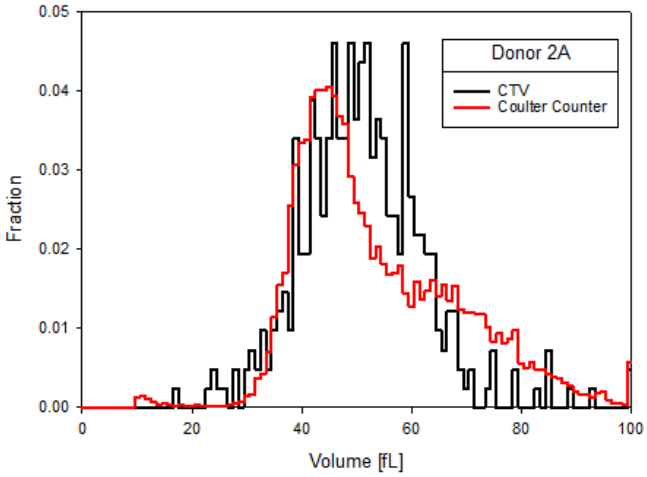

Figure 3. Volume distribution of RBCs measured on Coulter Counter and CTV for one sample.

Moreover, because of the disagreement in MCV values between CC and CTV and the normal range (within 80-100 fL), and since RDW is calculated from the MCV, the values of RDW_{CC} and RDW_{CTV} are slightly higher than the normal value (generally below 15%). Specifically, RDW is obtained based on both the width of the cell volume distribution and the average cell size (i.e. it is calculated by dividing the standard deviation of the mean cell size by the MCV of the RBCs). As can be seen in Table 1, the values reported by CC and CTV are slightly higher than 20%.

Hemoglobin Mass and Concentration in individual RBCs

The calculated MCH_{CTV} and $MCHC_{CTV}$ is represented in the scatterplot in **Figure 4** (for one of the donor samples, #1C). On the left, MCHC as a function of volume is presented, where the black dots correspond to the RBCs in the normal range compared to the red dots which correspond to the hypochromic RBCs. A high-volume hematology analyzer such as ADVIA® (Siemens Healthineers, Germany) uses spectral absorption reading to achieve similar parameters. **Figure 4** demonstrates that our CTV system can measure the same parameters in a similar fashion, as thousands of RBCs are tracked individually per sample.

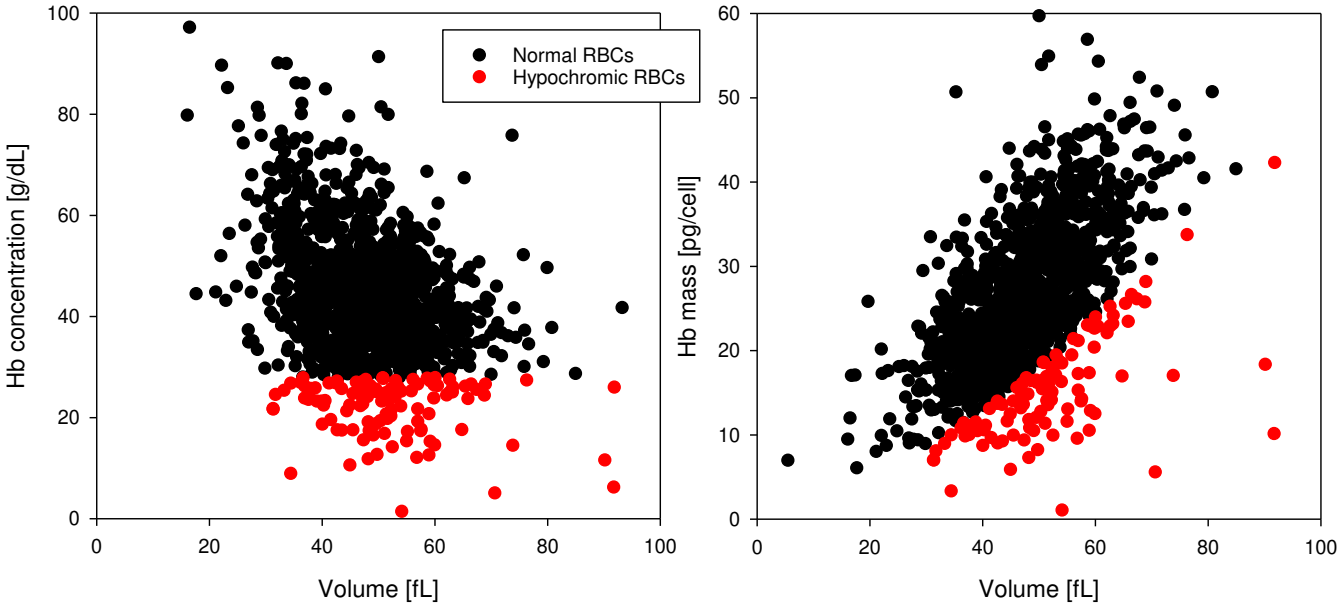

Figure 4. MCH_{CTV} and $MCHC_{CTV}$ measurements for one of the donor samples, showing the hypochromic RBCs in red.

By observing both **Figure 4** and **Table 1**, it can be concluded that the MCH (expressed in pg of Hb per RBC) values reported by CTV are within the normal range reported for healthy individuals, between 27 and 33 pg. Moreover, our previous study assesed the performance of CTV on measuring mass of Hb on indicidual RBCs by comparing CTV results to the standard method based on spectrophotometry, and our instrument reported accurate measurements/analysis [21]. Nevertheless, when comparing the MCHC measured by CTV to the reference range of 28-41 g/dL [23], CTV measured higher MCHC due to the fact that the measured MCV is smaller than the reference range, as happened to the RDW values from the CTV instrument.

Additionally, observing **Table 1** also shows that besides MCV, the overall Hb_{spec} of donor 6 is much lower compared to other donors, and the Hypo fluctuation is much greater compared to the samples obtained from donors 1-5. We have represented the temporal evolution of Hypo (%) for all the donors (blood was drawn from each donor on a weekly basis). It is known that during recombinant human erythropoietin (rHuEPO) therapy for anemia, Hypo > 10% is an indication for iron supplement requirement [16], and compared to other donors whose values stay around this threshold, the value for donor 6 fluctuates up to 55%. Hypo has been considered as a very sensitive marker because small changes in the number of RBCs with inadequate hemoglobin can be measured before there is any change in the MCHC [15]. Thus, quantification of hypochromic and/or hyperchromic red cells is helpful in the diagnosis of anemia. In fact, in a population of young anemic females, the percentage of hypochromic RBCs had the highest accuracy in distinguishing IDA from other anemias with normal iron stores [26]. Based on this, we concluded that our CTV can be very useful not only in measuring the mass of Hb in individual RBCs, but also on the diagnosis of the IDA.

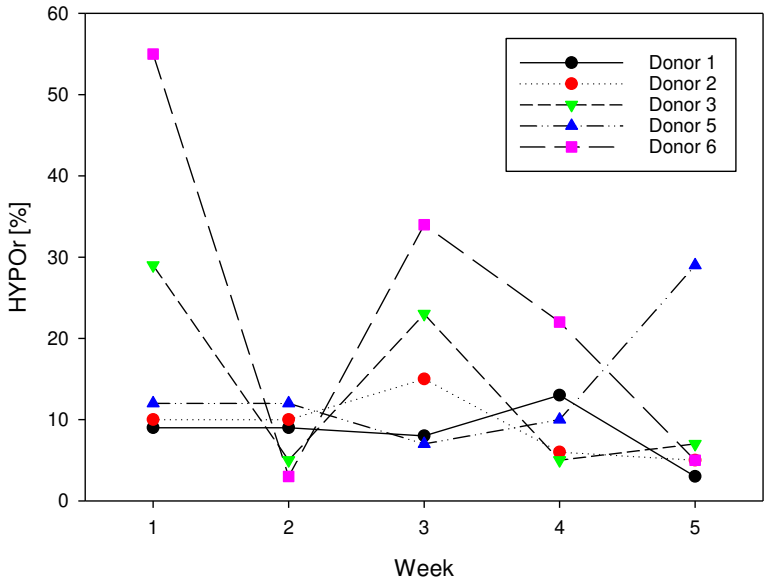

Figure 5. Temporal evolution of the percentage of hypochormic RBCs measured on CTV for all the donors.

Finally, and for further analysis on these donor's (donor 6) samples, the histogram of the weekly measured MCHC and MCH values has been presented in **Figure 6**. On the left side of **Figure 6,** the red line corresponds to the hypochromic RBCs and the black line presents normal RBCs in terms of Hb concentration. As expected, the normal RBC population decreases as the Hypo percentage increases and vice versa. One interesting fact is that while the overall Hb concentration stays around 12.7-12.9 g/dL, the ratio of the hypochromic RBCs and normal RBCs seems to have a dynamic fluctuation. For comparison, the histogram of Hb mass is presented on the right side of **Figure 6**, showing a similar fluctuation to the MCHC presented on the left side of **Figure 6**.

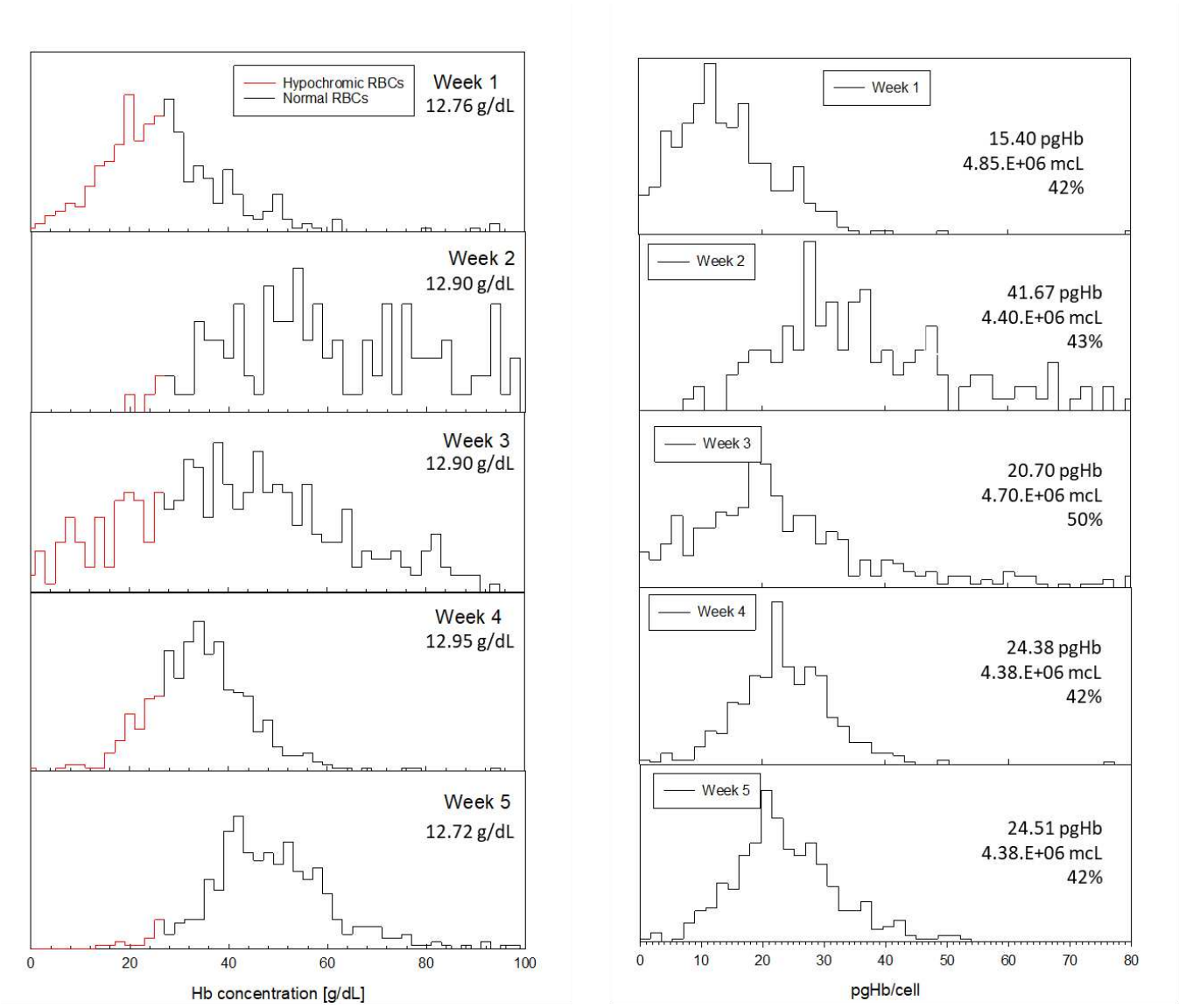

Figure 6. Weekly measurements of MCHC and MCH on CTV for donor 6.

CONCLUSION

Over the last few years, several studies have emphasized the global concern about the prevalence of iron deficiency anemia (IDA) or its precursor state, iron deficiency, in both developed and developing countries. In fact, reducing anemia by 50% in women is a 2025 World Health Assembly Global Nutrition Target [29]. Detection of anemia in state-of-the-art hospitals and clinics can be easily performed through complete blood counts. However, most of hematology analyzers currently available are large instruments, are not economical or portable and often require trained technicians. Thus, there is a need for a portable and economical hematology analyzer that can be use at the point of need, such as at mobile blood donation platforms for screening purposes. For example, it has been stablished that the Hb of a blood donor drops by 1–1.5 g/dL after donating a single unit of whole blood [30]. Thus, an appropriate pre-donation test may mitigate the possibilities of rendering the blood donor anemic, which

has also an explicit implication on the recipient's health. However, no testing methodology and sample requirement have been specified for Hb screening [30].

In this work, we evaluate the performance of a system referred to as the cell tracking velocimetry, CTV, to measure several hematological parameters from fresh human blood obtained from healthy donors. This system is based on the paramagnetic behavior that methemoglobin containing RBCs experience when suspended in water after applying a magnetic field. CTV uses a combination of magnets and microfluidics and has the ability to track the movement of thousands of red cells; thus, unlike other portable analyzers, CTV is able to measure not only traditional RBC indices but also novel parameters that are only available for analyzers that assess erythrocytes on a cell by cell basis. As such, we report for the first time the use of our CTV as a hematology analyzer able to measure MCV, MCHC, MCH, RDW or the percentage of hypochromic RBCs.

Our results indicate that most of the parameters measured with CTV are within the normal range for healthy adults, especially MCH. However, measures of the RBC volume (and thus, parameters related to volume such as MCHC and RDW) are outside the normal range; both CTV and CC reported MCV values that are almost half of the value for a normal adult. The reasons for these discrepancies are attributed in part to the plasma entrapment when measuring Hct (used to calculate the MCV by the traditional method) and also to the deformability of red cells, which is not taken into account when measuring MCV by CC and CTV. Since no internationally accepted reference method has been published for measuring MCV, we conclude that CTV could potentially be employed for measuring MCV along with other hematological parameters such as MCH, MCHC, RDW and hypochromic red cells, as long as instrument-specific reference ranges are stablished or correction factors are applied, as happens with several commercial analyzers. Comparing the resolution and accuracy of our system to other hematology analyzers, along with its portability, price and fast measurement (less than 5 minutes for tracking thousands of metHb-RBCs), we believe that CTV is a promising technology to be used for blood testing at the POC or in low resource areas. Potential applications include: population screening for anemia, assessing the eligibility of blood donors, triage of trauma victims, and perioperative assessment of a patient's transfusion needs, which will facilitate real-time clinical decision making.

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ACKNOWLEDGEMENTS

We wish to thank the National Heart, Lung, and Blood Institute (1R01HL131720-01A1) and DARPA (BAA07-21) for financial assistance.

AUTHOR CONTRIBUTIONS

J.G.P., J.K., and M.W. developed the experimental plan and performed the experiments. A.F.P. and M.Y. helped with the analysis of the results. P.C.D., M.Z. and J.J.C. provided technical guidance and reviewed final results. All authors reviewed the manuscript.

ADDITIONAL INFORMATION

Competing Interests: The authors declare no competing interests.

Figures

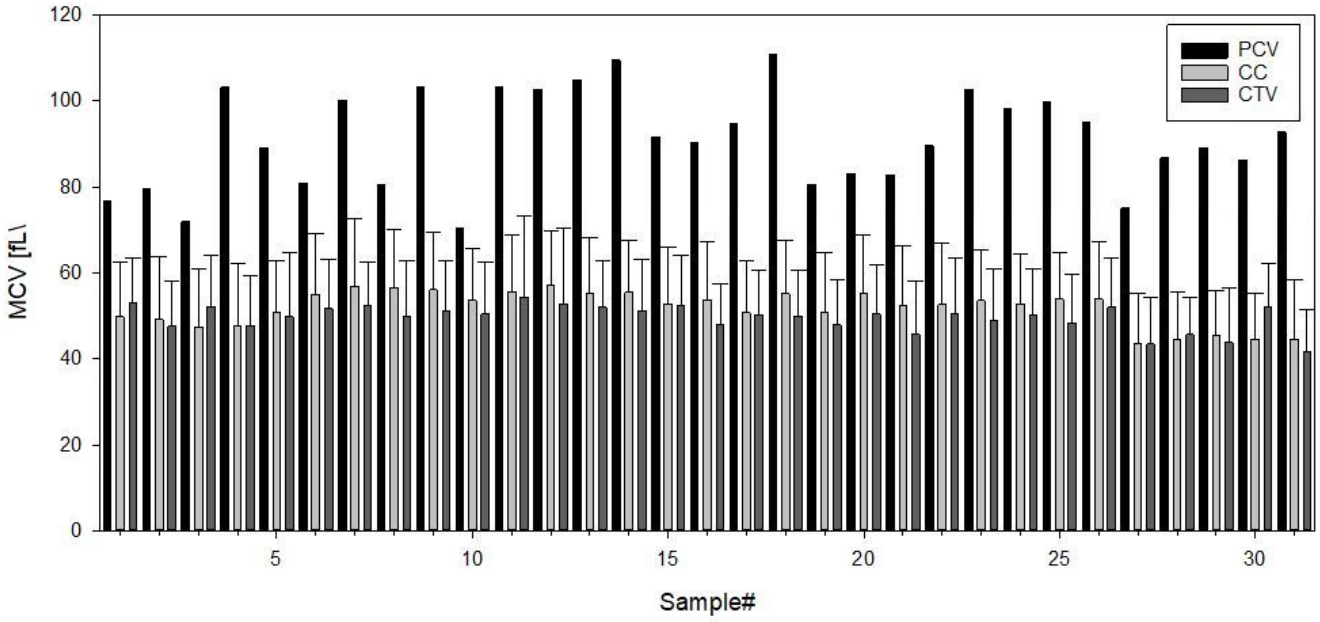

Figure 1

Comparison between the MCVCC, MCVCTV and the MCVave value calculated from the Hct obtained by the PCV method for the total 31 samples.

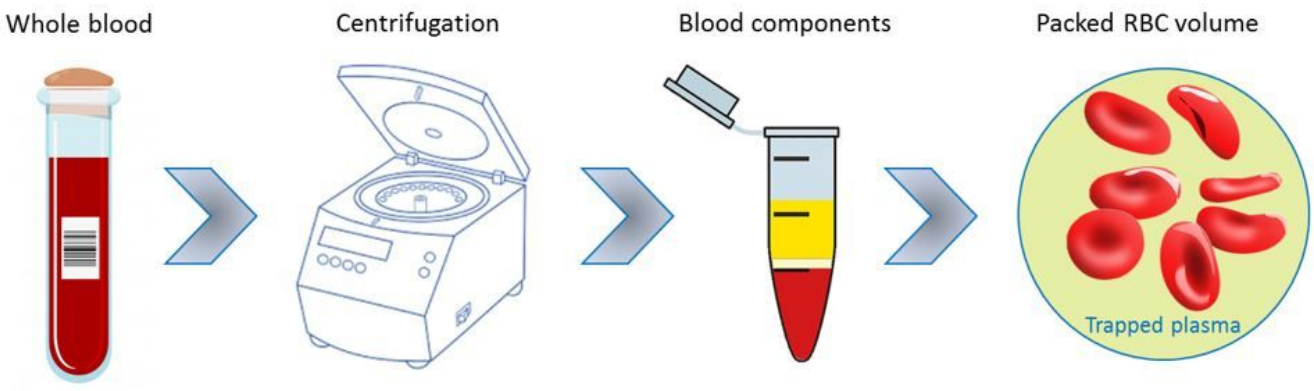

Figure 2

Schematic representation of plasma entrapment during Hct measurements by the PCV method.

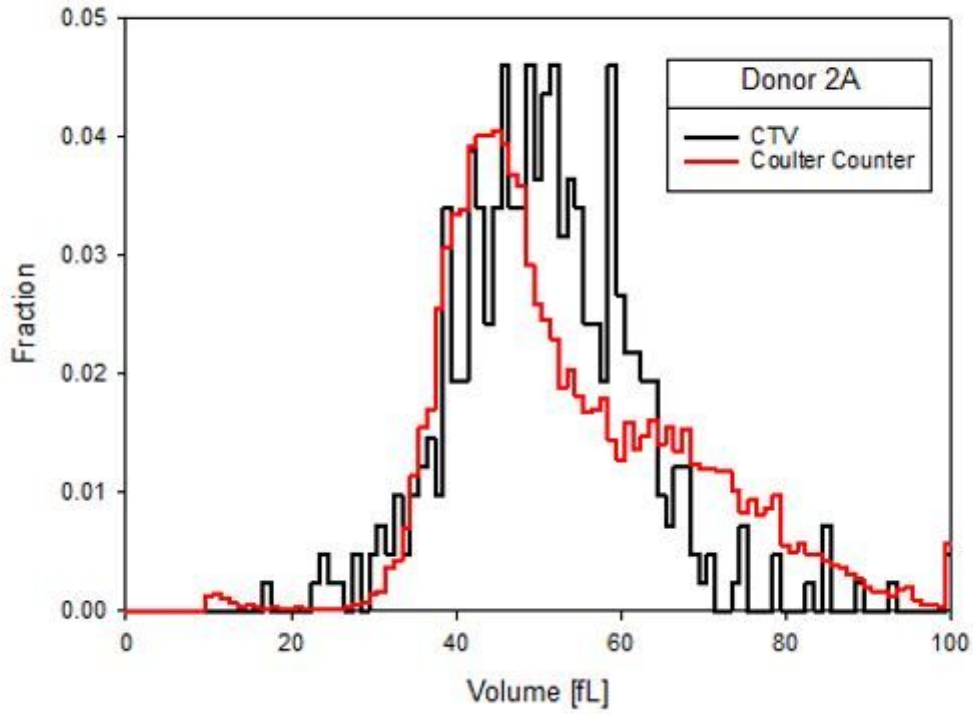

Figure 3

Volume distribution of RBCs measured on Coulter Counter and CTV for one sample.

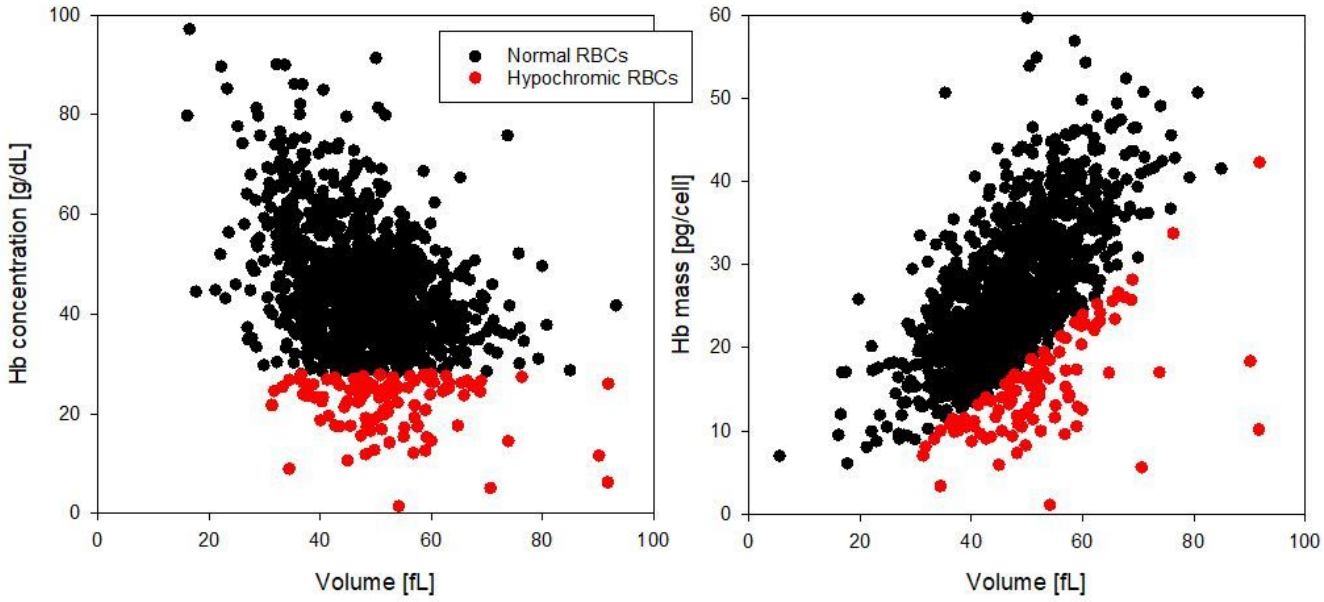

Figure 4

MCHCTV and MCHCCTV measurements for one of the donor samples, showing the hypochromic RBCs in red.

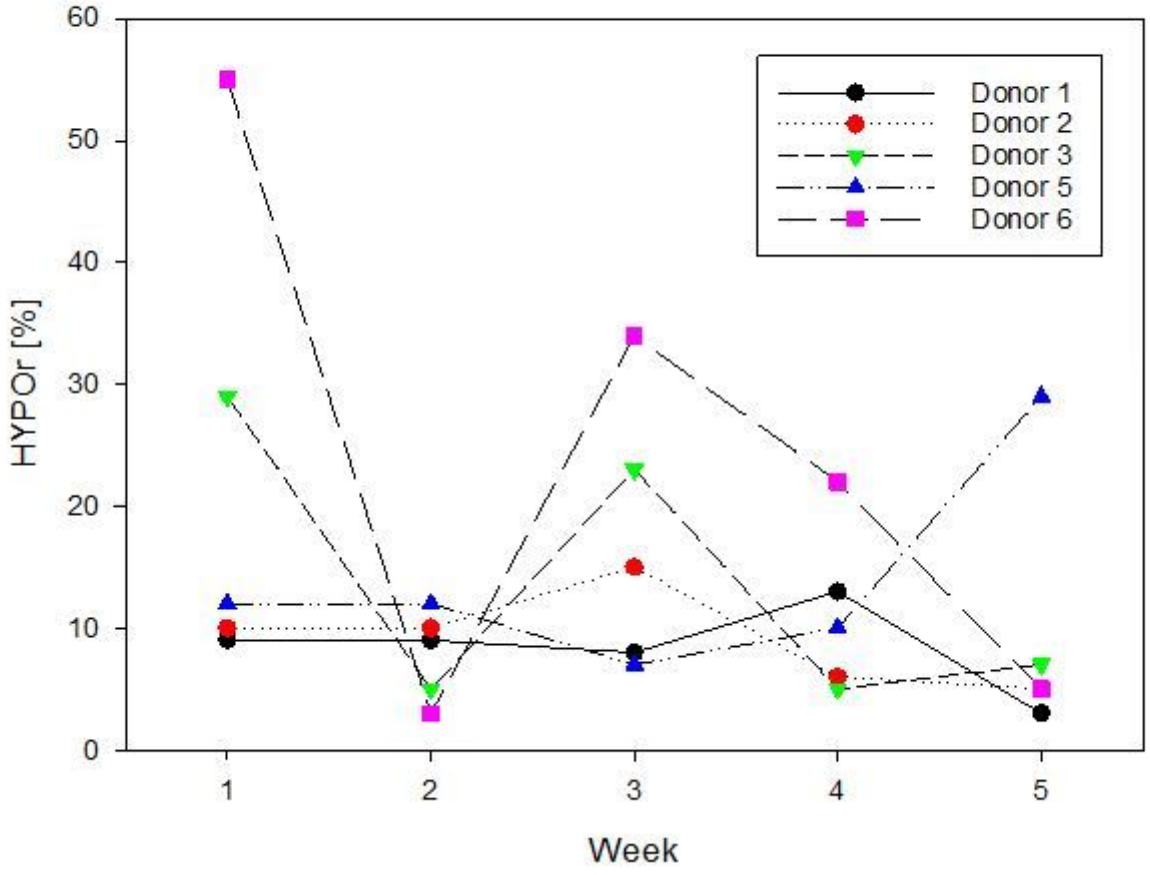

Figure 5

Temporal evolution of the percentage of hypochormic RBCs measured on CTV for all the donors.

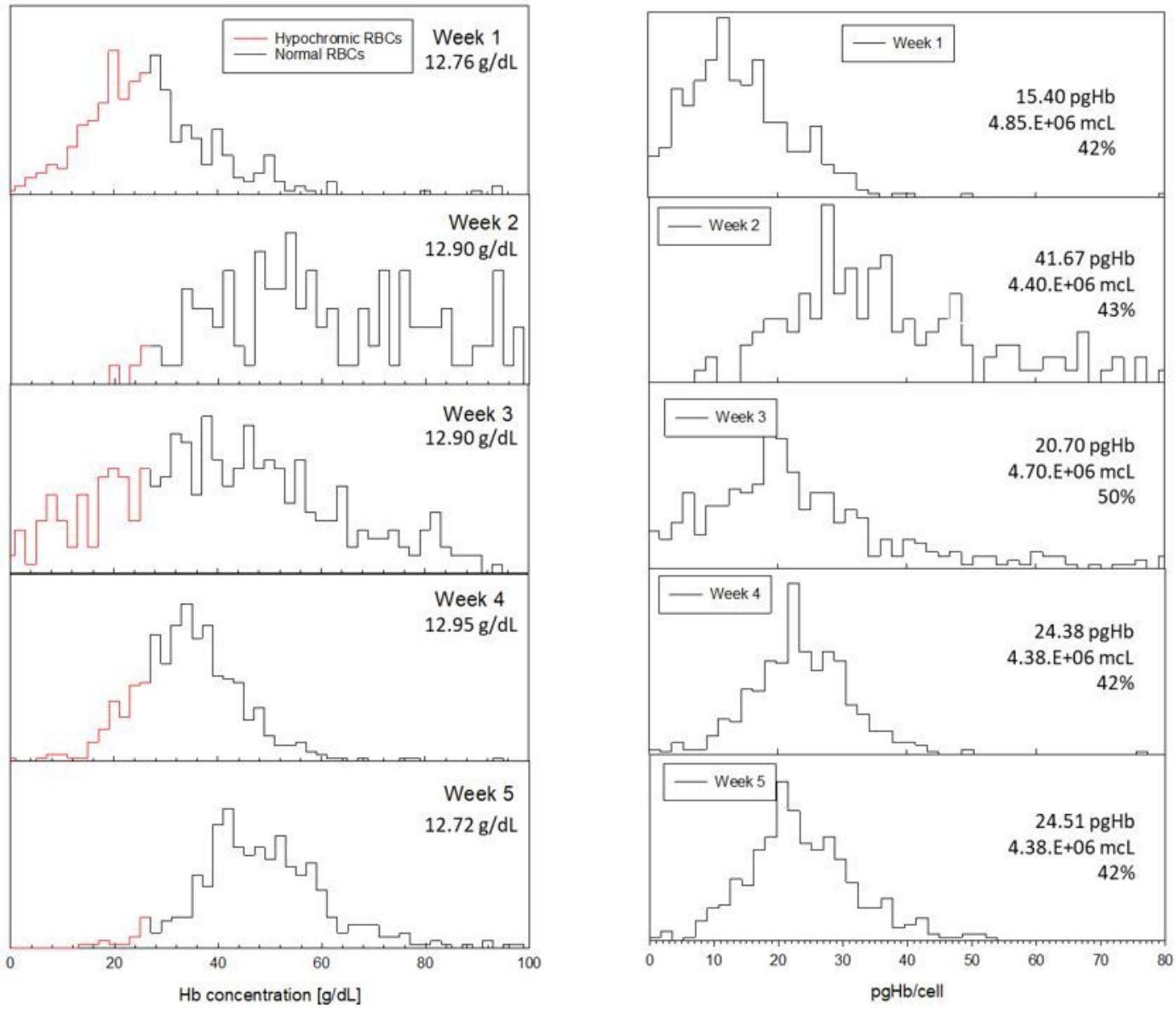

Figure 6

Weekly measurements of MCHC and MCH on CTV for donor 6.

Supplementary Files

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