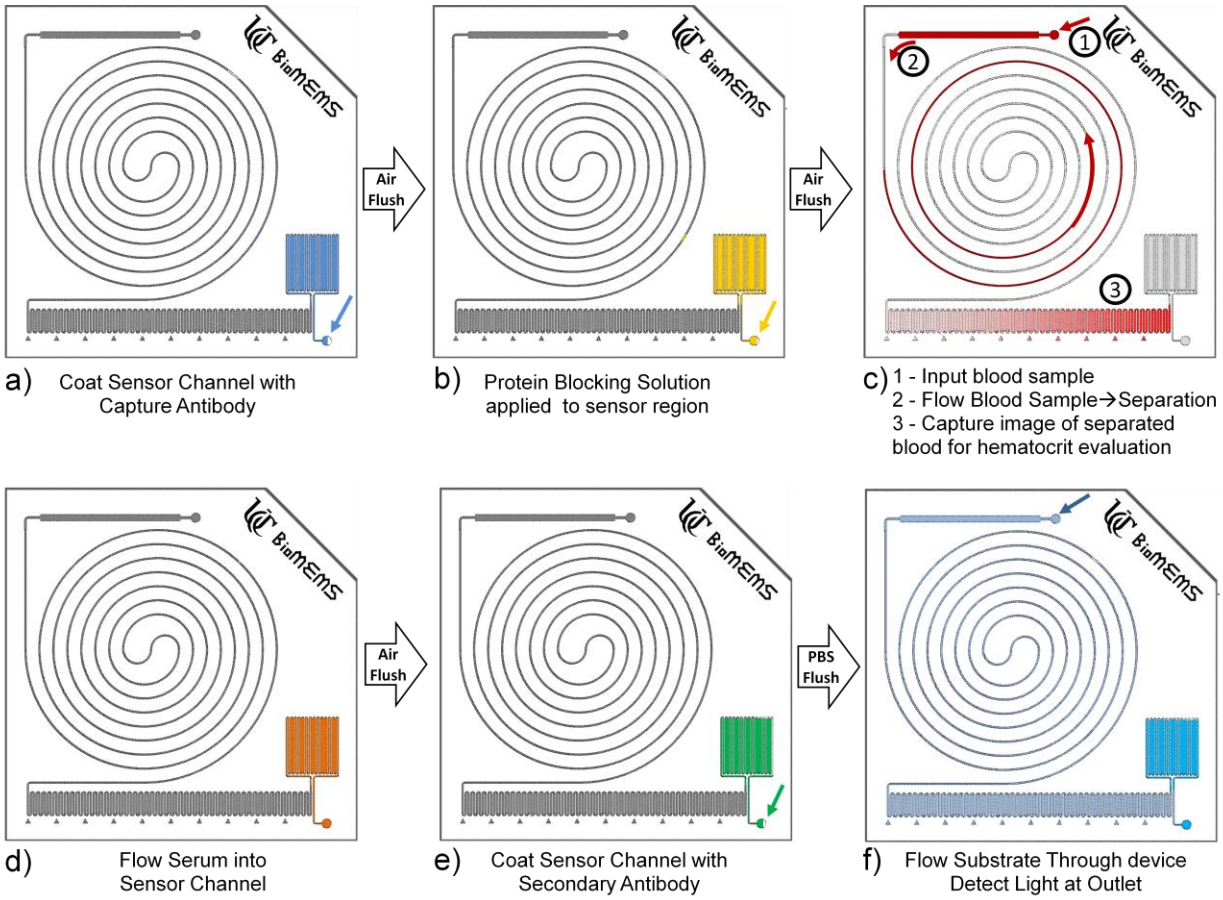
Supplemental Data and Figures

Browne et al. **"A lab-on-a-chip for rapid blood separation and quantification of hematocrit and serum analytes."**



Supplemental Figure 1: *Integrated blood analysis chip preparation and operation sequence. a)Preparation of the blood analysis chip begins with coating the ELISA sensing region with a primary capture antibod. b) The capture antibody is flowed out of the sensing region and protein blocking solution is flowed into the sensing region to block regions of the sensor channel not covered with capture antibody in order to avoid non-specific binding of secondary antibody in later steps. The protein blocking solution remains in the sensor channel until the chip is used. c) When a sample of blood is flowed into the chip's inlet air in the separation and hematocrit evaluation channels displaces the protein blocking solution and the blood is flowed until it occupies the entire volume of the hematocrit evaluation channel. At this point an image of the blood in the hematocrit evaluation channel is captured by a point-and-shoot camera and gray scale analysis performed to evaluate the length of the packed cell portion of the sample. d) The blood sample is then advanced into the high surface area to volume ELISA channel until the rear of the blood sample which has been depleted of red blood cells occupies the antibody coated ELISA channel.The cell depleted plasma is incubated for 2 minutes to allow optimal antibody-antigen binding. e) Following incubation of the sample in the ELISA channel, peroxidase-conjugated detection antibody is flowed into the outlet to occupy regions of the ELISA channel where target antigen has been immobilized. f) In the final step, substrate for the detection antibody is flowed into the chip inlet, light produced in the ELISA channel detected by a photodetector and signal produced correlated to sample concentration.*

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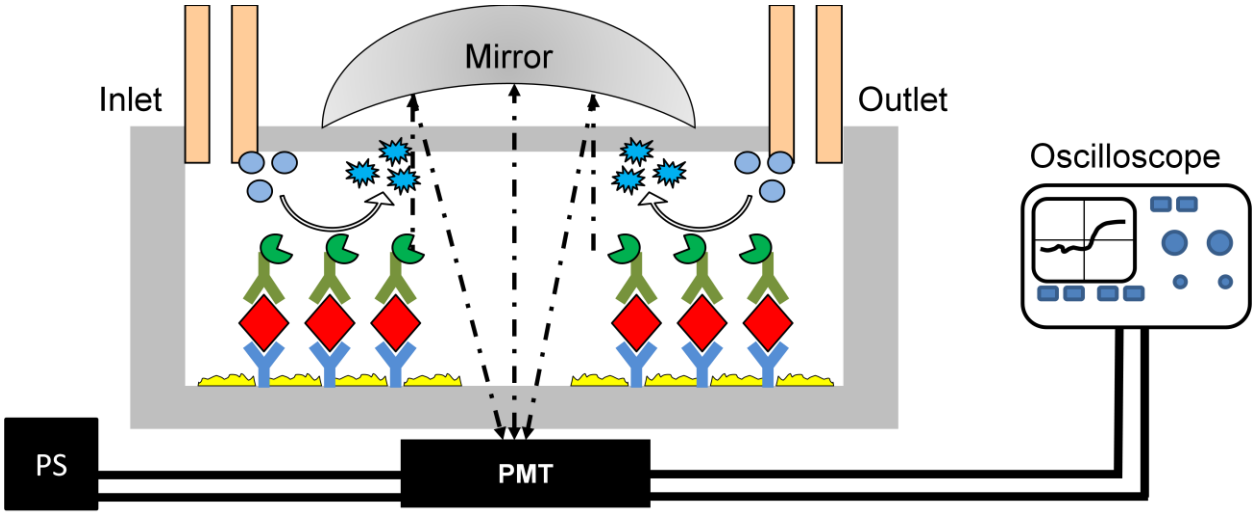
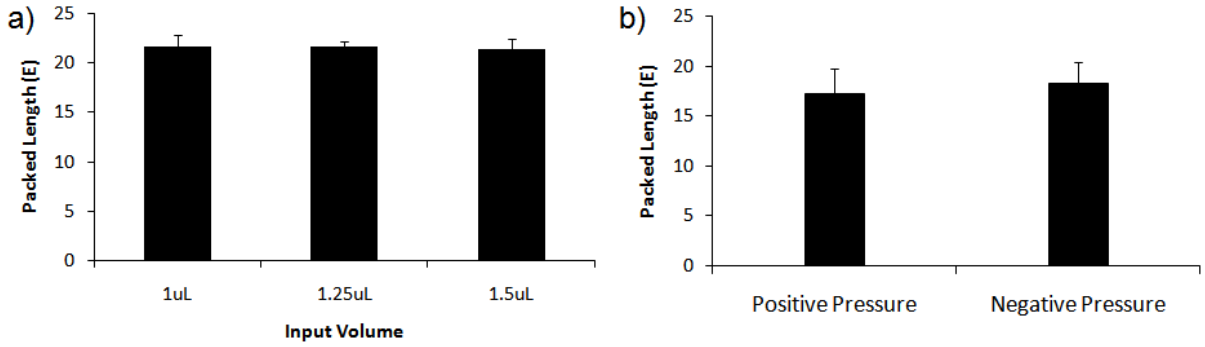


Supplemental Figure 2: Experimental setup for ELISA luminescent detection. Immobilized ELISA reagents in a microchannel generate luminescent light when substrate is flowed through the device inlet. Light is reflected by a 9mm concave mirror (Edmund Optics) and collected by a Hamamatsu H5773 photomultiplier tube (PMT,). The PMT is powered by a Tenma 72-5526 DC regulated Power Supply (PS) and voltage signal proportional to light collected by the PMT is logged and displayed by an Agilent Technologies DS0534A oscilloscope.



Supplemental Figure 3: Separation parameters with statistically insignificant effects on separation. a) Effect of input volume on packed cell volume E (Hematocrit = 41%). b) Effect of flow pressure on packed cell volume E (Hematorcrit = 38%).

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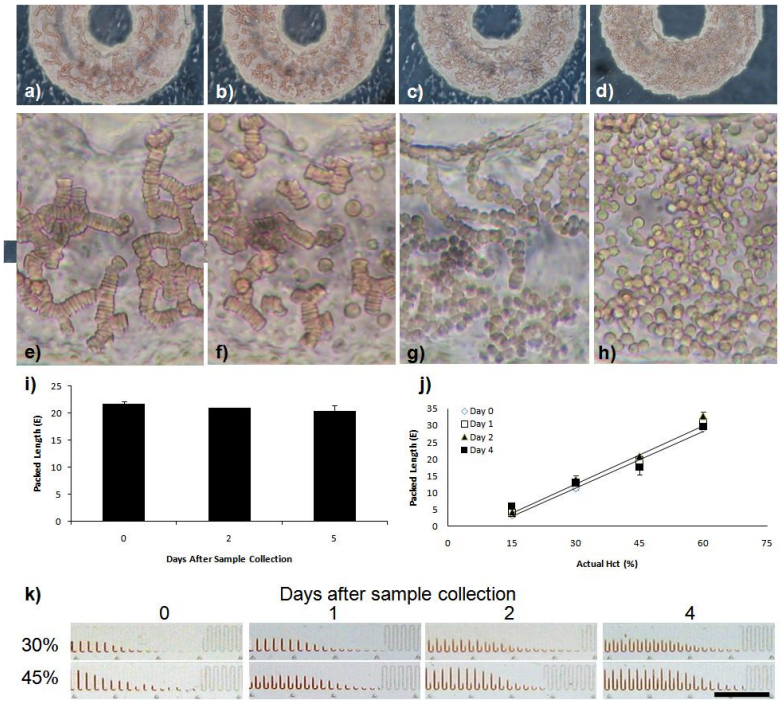
Effect of sample age and erythrocyte aggregation on separation

For LOCs to gain clinical practicality they must function reproducibly for all patients. We were initially concerned that patients with erythrocytes demonstrating differing aggregation characteristics would alter the separation dynamics resulting in aggregation dependent hematocrit values. The formation of rouleaux in microchannels and *in vivo* microcirculation has been well documented [39-43]. Because rouleaux are stacks of red blood cells that can form large branched networks, we initially thought that rouleaux size might affect flow dynamics and subsequently devalue the hematocrit evaluation technique developed in this work. However, we observed that fresh blood samples aggregated to form rouleaux during flow, while older samples did not form rouleaux. Ultimately, erythrocyte aggregation had no effect on hematocrit evaluation and an indiscernible effect on degree of cell-depletion in separated plasma. The details of these observations are included in supplemental data with.

Methods: One microliter of blood was evaluated for hematocrit via flow based separation on the same day as it was collected and on subsequent days. **Supplemental Figure**4a-h are low and high power photomicrographs of cell depleted plasma toward the rear of separated samples processed on different days after sample collection. Robust rouleaux formation was seen when the image was captured immediately following flow-based separation on the same day as blood sample collection (**Supplemental Figure 4**a and e). Images were captured of the same region as those captured on day 0 (**Supplemental Figure 4** a and e) one day (**Supplemental Figure 4** b and f) and 3 days (**Supplemental Figure 4** c and g) after flow-based separation was performed on a fresh blood. Over time it is apparent that rouleaux that formed on day 0 slowly disaggregated until 3 days after separation when rouleaux appeared more like a string of beads than a stack of coins. To compare the effect of erythrocytes that do not aggregate with those which do, 3 day old blood samples were subjected to flow-based separation and images were captured of cell depleted plasma (**Supplemental Figure 4**d and h). These observations show that erythrocytes in fresh blood samples aggregate to form rouleaux while erythrocytes in the same sample that is subject to flow based separation 3-days following sample collection do not form rouleaux.

We subsequently evaluated hematocrit for fresh and older blood samples to determine whether erythrocyte aggregation affects hematocrit evaluation technique developed in this work. In **Supplemental Figure 4**i a native blood sample was separated and packed cell length evaluated on days 0, 2 and 5. **Supplemental Figure 4**j plots data for a blood sample that was partitioned into a range of hematocrits and evaluated on days 0, 1, 2 and 4. Both **Supplemental Figure 4** i and j demonstrate the statistically insignificant variability of packed cell length for blood samples that are separated on the same day those collected on subsequent days. The cell-depleted region of samples with hematocrits of 30 % and 45 % are shown for images captured immediately following flow-based separation and subsequent overnight gravity separation as described earlier (**Supplemental Figure 4**k). Visual inspection of flow-separated samples that were subsequently subject to gravity-driven separation demonstrate minor differences in erythrocyte content between samples that do and don't aggregate (fresh and older samples respectively). Future study would be necessary to evaluate whether minor differences in erythrocyte content of cell depleted plasma has an effect on protein quantitation of cell-depleted plasma samples produced by flow-based separation.".



Supplemental Figure 4: Effects of sample age on hematocrit evaluation and serum separation. a-h) photomicrographs of blood samples processed by flow based separation. a,e) Blood sample separated on the same day it was collected showing robust rouleaux formation; Images of the separated blood sample imaged in a and e were taken 1 day (b,f) and 3 days after separation (c,g) showing disaggregation of rouleaux; d,h) Blood sample separated 3 days after sample collection showing complete absence of rouleaux formation; i) Packed cell length evaluation for a 1 mL blood samples pased through microfluidic chip on the same day it was collected and 2 and 5 days after collection. j) Hematocrit evaluation for blood sample partitioned into different hematocrits and processed through microfluidic chip on day 0, 1, 2 and 4. k) Rear of separated blood sample for hematocrit of 30% and 40% indicating minor changes in erythrocyte content over time (scale bar is 3mm). Channel width is 150 μm in all figures.

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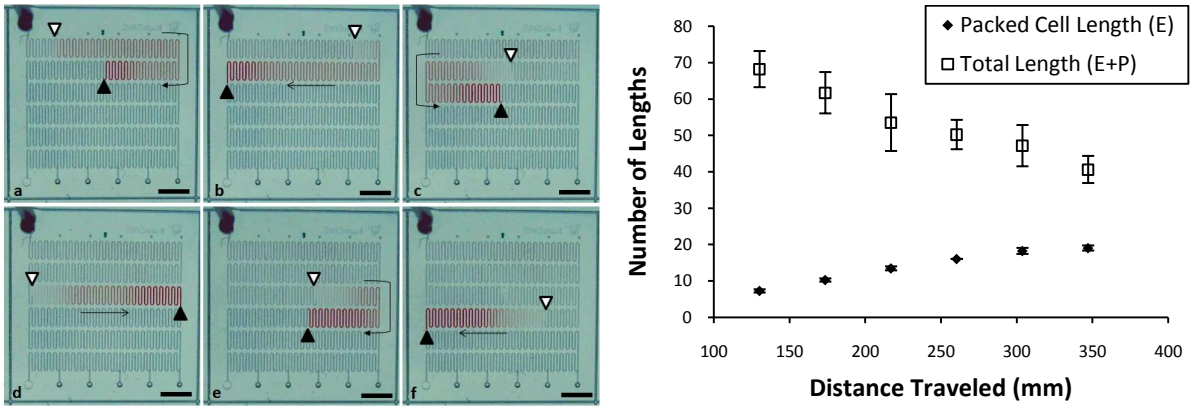

Supplemental Figure 5: Evaluation of dynamics of blood separation with distance. a-f) Sequential photographs of 0.5 mL blood sample as it flows through the long serpentine channel (150 mm). Black triangle indicates the flow front, white triangle indicates sample rear and arrow indicates flow direction. Scale bars are 3mm long. g) Plot of cell packed length (E) and sample length (P+D) (average of 5 trials) as the sample flows through long serpentine channel.