Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Additional Methods

The HYPER pad fabrication

The HYPER pad has a novel structure including differentiation pad (FR-01), a filtration pad (Vivid Plasma GF) and a calibration pad (Fusion 5) to perform high-yield blood cell separation. Fig S1 shows in detail the fabrication methods of the HYPER pad. In the preparation step, FR-01 pad, GF membrane were cut to 300 mm long, 12 mm and 8 mm wide strips respectively. The Fusion 5 membrane was cut to 300 mm length, and desired width according to the correlation between the length of calibration pad and the outflow serum volume. The HYPER pad is fabricated follow the steps below.

1. Place two a piece of plastic tape (3M) on workbench, sticky side up. Leave 8 mm space between them, as shown in Fig. S1a.
2. Attach the FR-01 to the two pieces of the plastic tape, with 1 mm overlap to the tape in the top and 3 mm overlap to the tape in the bottom, as shown in Fig. S1b.
3. Attach the GF membrane, with a 7 mm overlap with the FR-01 membrane, as shown in Fig. S1c.
4. Attach the Fusion 5 membrane, with a 7 mm overlap with the GF membrane, as shown in Fig. S1d.
5. Fold the plastic tape on the top side to ensure the attachment between the filtration membrane (GF) and the calibration pad (Fusion 5), as shown in Fig. S1e.
6. Cut into desired width. All tests performed here used 4 mm wide HYPER pad (Fig. S1f).

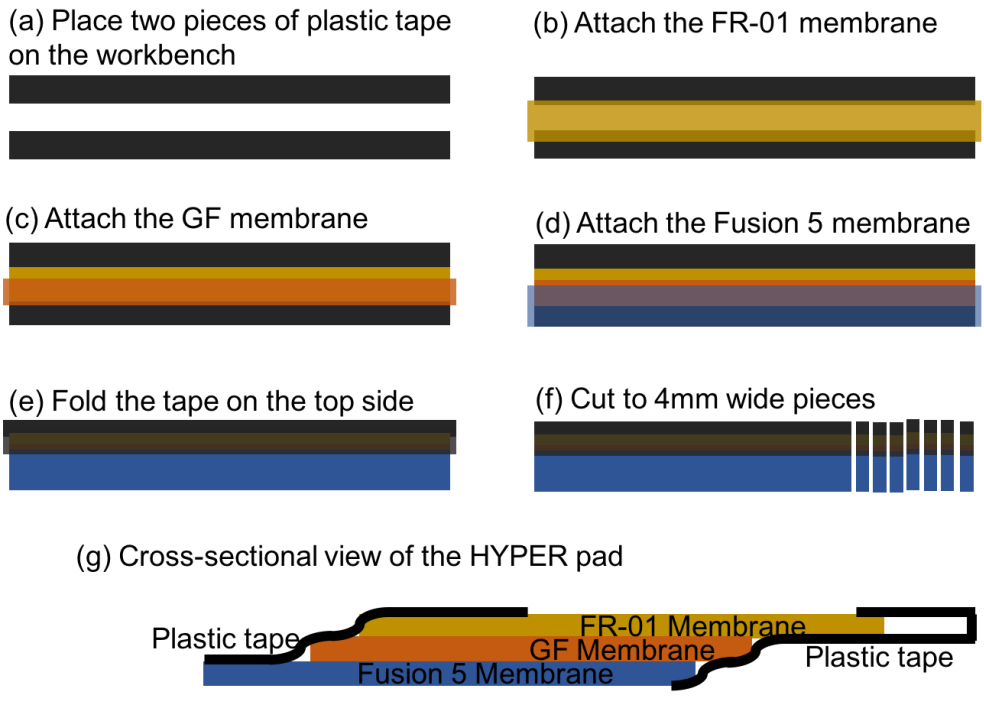

Figure S1. Detailed fabrication method for the HYPER platform (a-f) Detailed method for fabrication of the HYPER platform. (g) The Cross-sectional view of the HYPER pad.

The cross-sectional view of the HYPER pad is shown in Fig. S1g. The plastics tape holds all membranes together. Since all layers in the HYPER pad are porous media made of hydrophilic material, serum intended to fill them up. This property allows extra tolerance in the fabrication process, because air can exit through the pores in the media, and eventually the surface tension of serum will hold each layer together.

Filtration membrane characterization section

The filtration membrane is made from Vivid Plasma Membrane GF (Pall Corporation) that the estimated mean flow pore for the filtration membrane is 1.9 ± 0.4 μm, and goes from an estimated 50 μm to a 2 μm. We characterized the filtration membrane, and measured the pore size by ourselves with Scanning Electron Microscope (SEM). We presented the SEM microscopy image of the membrane from the side at 400X, 1000X and 4000X magnifications, as well as 1000X magnified image of the intersection that shows the descending pore size of the membrane in Fig 8. The SEM figures agree with the manufacturer's claim on the membrane specification, The image is obtained with Zeiss Ultra SEM, with Extra High Tension (EHT) voltage at 0.7 kV and the Secondary Electron (SE) detector.

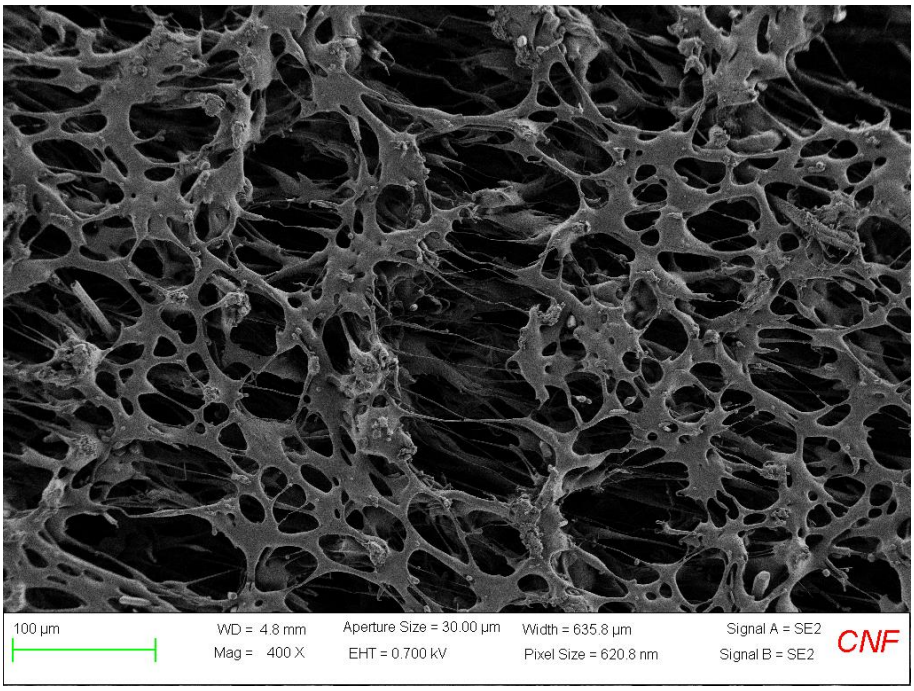

Fig. 2 SEM image of Vivid Plasma Separation membrane, blood entrance side, 400X

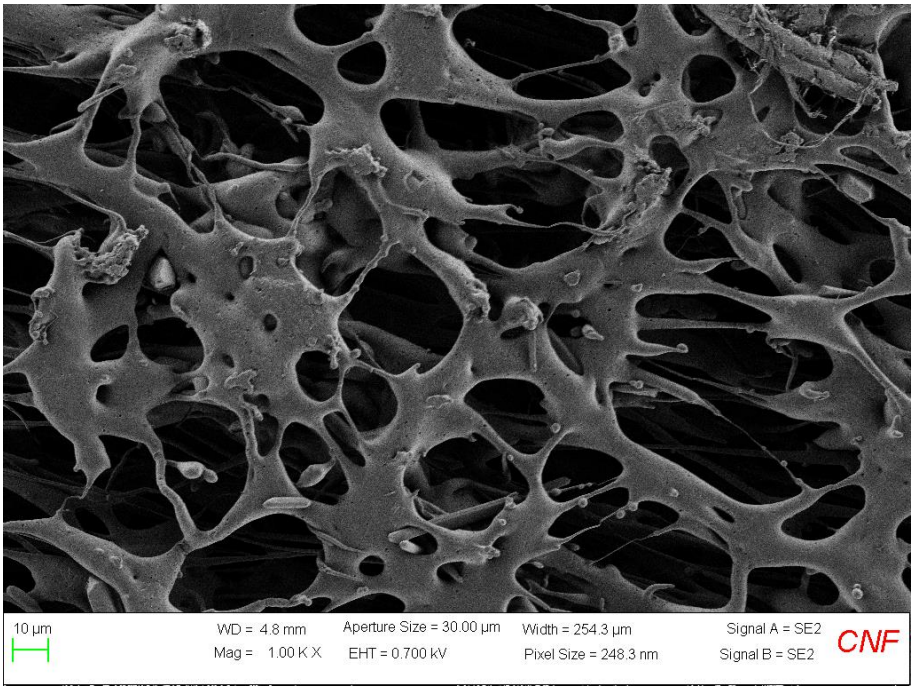

Fig. 3 SEM image of Vivid Plasma Separation membrane, blood entrance side, 1000X

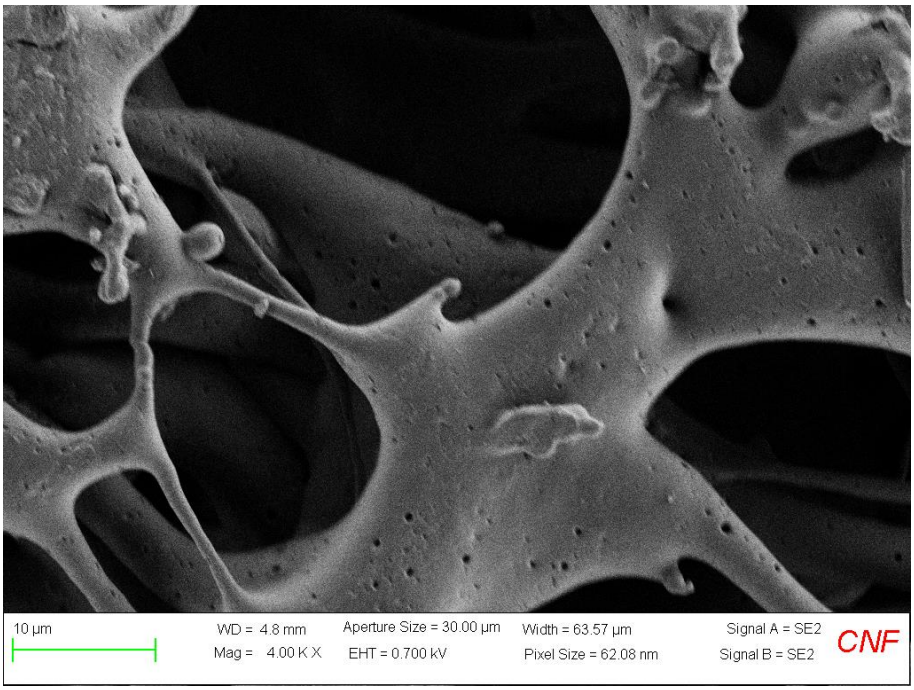

Fig. 4 SEM image of Vivid Plasma Separation membrane, blood entrance side, 4000X

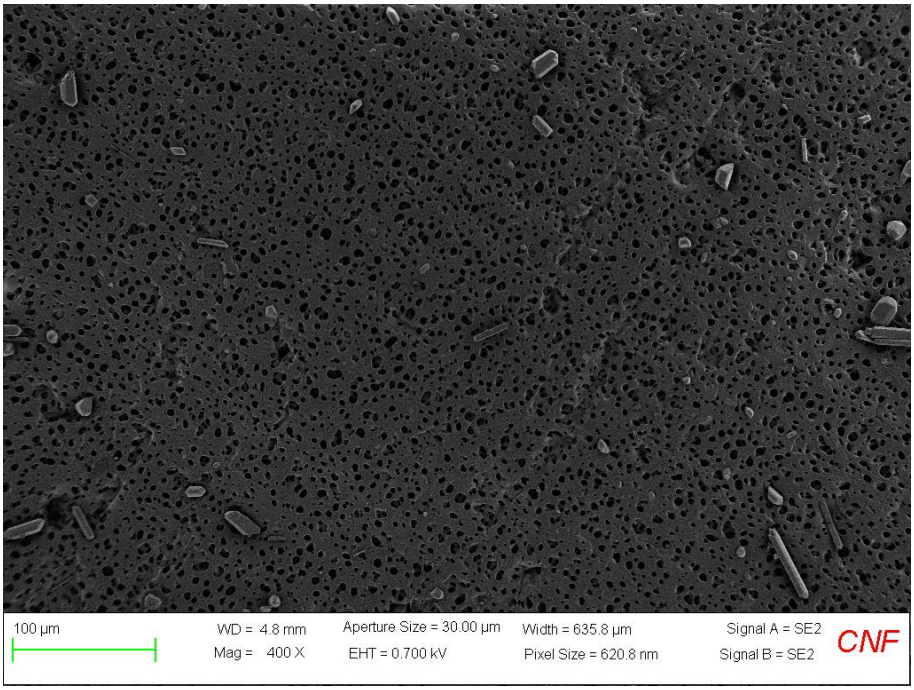

Fig. 5 SEM image of Vivid Plasma Separation membrane, blood exit side, 400X

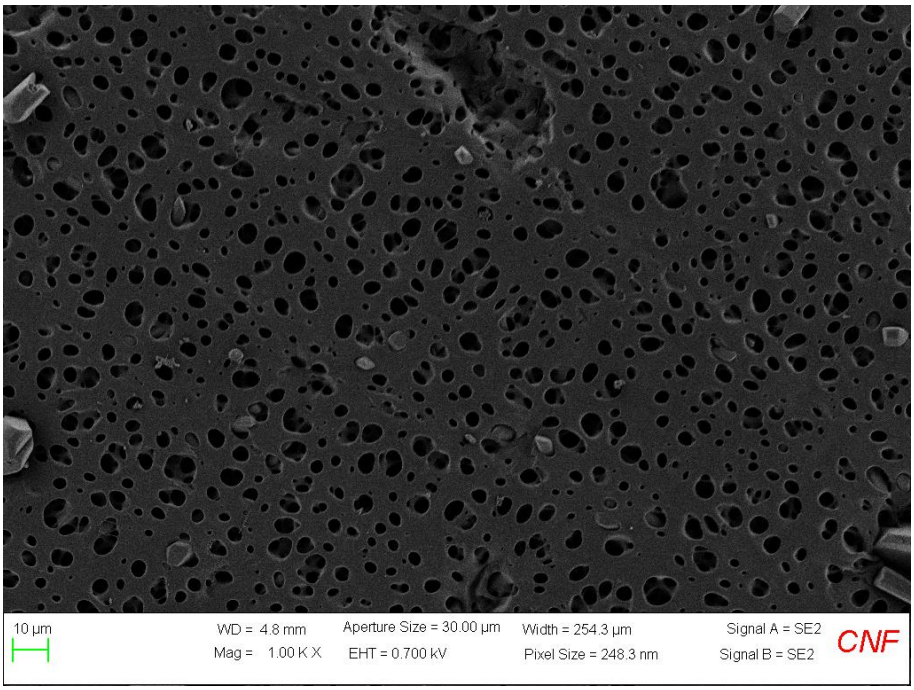

Fig. 6 SEM image of Vivid Plasma Separation membrane, blood exit side, 1000X

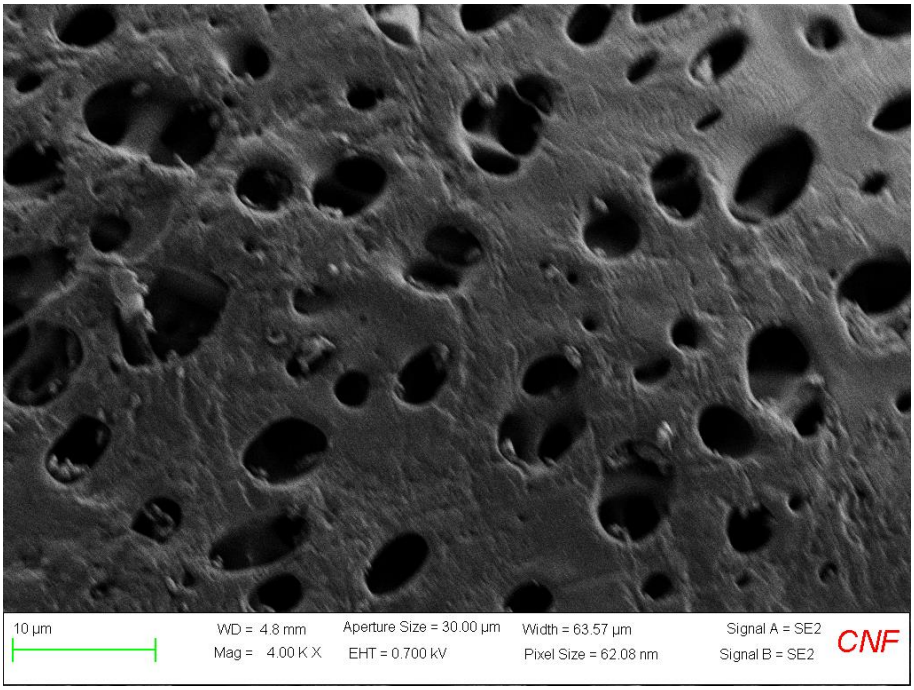

Fig. 7 SEM image of Vivid Plasma Separation membrane, blood exit side, 4000X

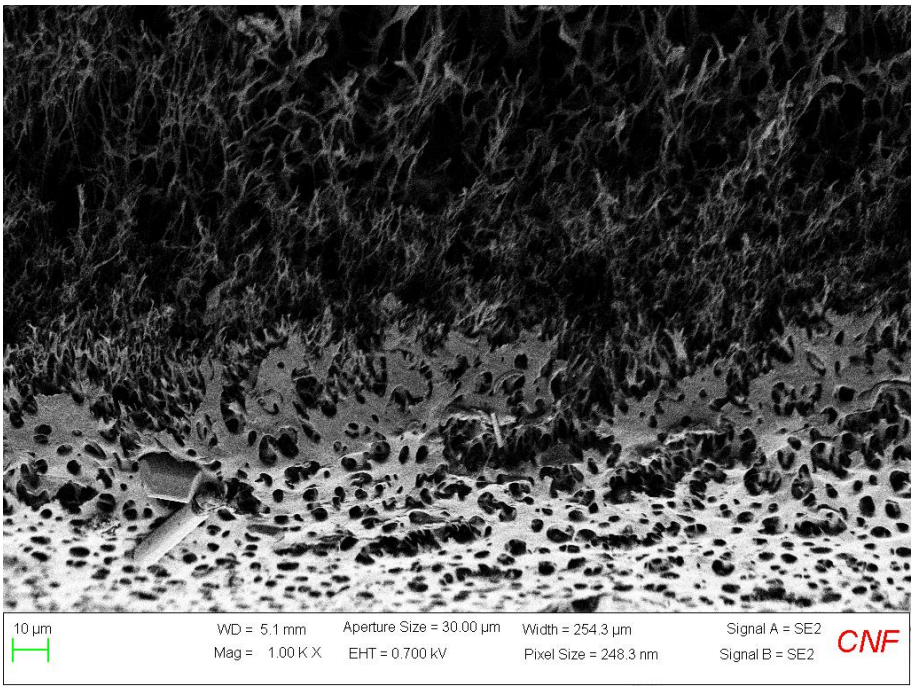

Fig. 8 SEM image of Vivid Plasma Separation membrane, intersection, 1000X

HYPER performance with unmodified whole blood

In the main text, we modified hematocrit of blood sample to 45%, in order to obtain unified experimental condition. Addition to tests with modified whole blood sample, we tested the HYPER platform with directly collected vane blood samples from 6 donors. The hematocrits of the blood sample were measured with packed cell volume (PCV) method. The whole blood sample obtained has an average hematocrit at 42%, and the standard deviation is 3.6%. We ploted the average volume of serum as a function of time for this test in Fig S2. For each data point, 6 HYPER pads were tested, and the error bar represents the standard deviation. The experiment demonstrates that, for whole blood sample directly collected from participants' vane, the HYPER platform has a yield of 61.5% at 10 minutes, and a yield of 81.6% at 20 minutes.

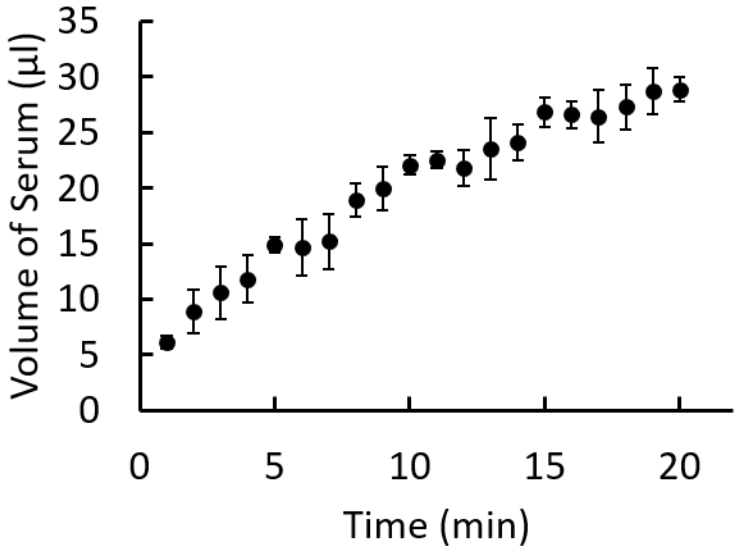

Figure S2. Performance of the HYPER platform with whole blood directly collected from participants' vane. Overall, the HYPER platform has a yield of 61.5% at 10 minutes, and a yield of 81.6% at 20 minutes. For each data point 6 HYPER pad were used and the error bar shows the standard deviation of 6 whole blood sample from different donors.

Evaporation loss measurement

Since the HYPER platform has a time-dependent separation efficiency and practically 5 – 15 minutes is required to achieve a high separation efficiency, it is necessary to measure the evaporation loss within in the separation process. To address the evaporation problem, we measure the weight change of HYPER pad when 80 μL whole blood was applied within a period of 30 minutes. The tests were performed indoor at 21℃ and 45% relative humidity. The result shows that the loss of serum due to evaporation is 4.1 μL and 7.6 μL, or 5.0% and 9.4% at the 15th minute and 30th minute respectively.

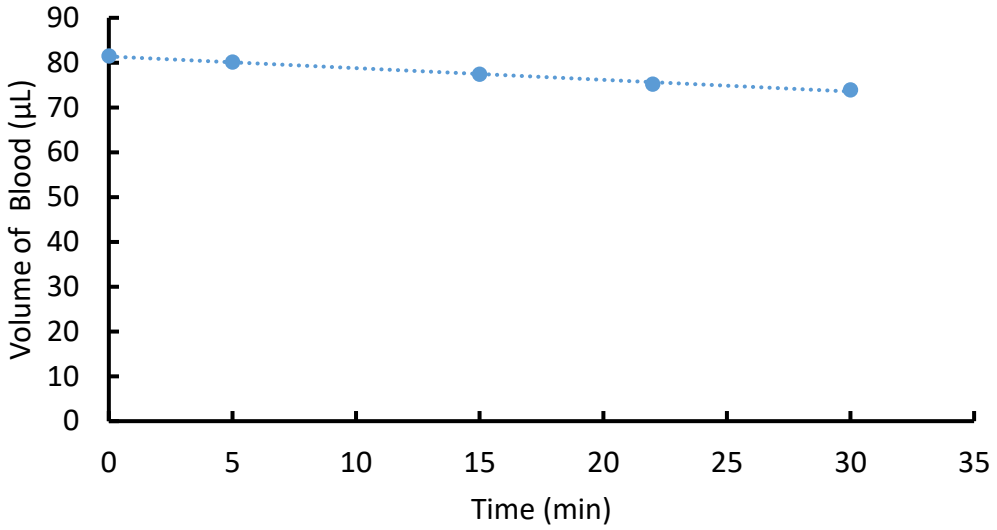

Figure S3. Effect of evaporation on the HYPER platform. When 85 μL whole blood is applied on the HYPER platform, the loss of serum due to evaporation is 4.1 μL and 7.6 μL, or 5.0% and 9.4% at the 15th minute and 30th minute respectively.

Fabrication of multiplex malnutrition tests

The HYPER platform extracts 16 μL clean serum out of whole blood sample with various hematocrit rate. The HYPER pad is integrated as the sample pad on the TIDBIT lateral flow immunoassay tests strip, which has polyclonal anti-ferritin IgG, RBP protein and polyclonal anti-CRP IgG as test lines, and anti-mouse IgG as control line dispensed on the nitrocellulose membrane. Monoclonal anti-ferritin, anti-RBP and anti-CRP IgG antibodies were conjugated with r-phycoerythrin (RPE), fluorescein (FITC), and phycoerythrin/cyanine 5 (PE/Cy5) respectively, and then preloaded on the calibration pad. The multi-color fluorescence immunoassay has balanced test line intensities for a larger physiological range of measurement. It also provides advantage in recognizing cross-binding between biomarkers. After the immunoassay is performed, ferritin and CRP concentrations are measured with sandwich type immunoassays, while RBP concentration is measured with a competitive immunoassay. The HYPER pad in this application, has an 8 mm long calibration pad. The calibration pad with such size is designed to measure 16 μL clean serum sample.

To run a test, first we applied 60 μL of whole blood sample on the differentiation pad and start the separation process. After 5 minutes, when the calibration pad absorbs ~16 μL serum, we remove the differentiation pad and filtration membrane, and apply 60 μL of running buffer to initialize the lateral flow immunoassays. We then use our previously developed TIDBIT reader to measure the fluorescence signal intensity on the

test strips. After 15 minutes, the ferritin, RBP and CRP test line shows orange, green and red color respectively. The fluorescence sensor in the TIDBIT reader takes image of the test strips, and average intensity of each test line is recorded as "brightness number" after background removal. Detailed methods in biomarker concentration measurement and processing is included in Supplementary Information.

12 whole blood samples from US adults were directly used as input in this study. After each test is done, the fluorescence signal intensity on the test strips are compared to the concentration of biomarkers characterized with laboratory standard ELISA methods, and fit to four-parameter logistic curves marker such that [marker] = f[brightness#]. Each data point represents 3 test strips using whole blood sample from the same participant, and error bar shows the range of fluorescence intensity.

HYPER pad performance and quantitative separation mechanics

The HYPER platform is especially suitable for accepting finger-prick blood sample, which have various amount of whole blood sample. As a proof, we determined the range of whole blood sample volume for separating certain amount of serum, as shown in Fig 4d. The recommended amount of whole blood sample is the average of maximum and minimum volume of whole blood sample that gives desired amount of serum. The error bar shows range of whole blood required. If inflow whole blood sample falls within the error bar range, the HYPER pad will separate an amount of serum that is within 1±5% of the desired volume in 10 minutes.

Fig 9 shows the quantitative blood separation mechanics step-wise. At the beginning of the separation process (step 1), the whole blood sample over-saturates the differentiation pad, so serum is released to the calibration pad. After about 5 minutes (step 2), the calibration pad has absorbed a specific amount of serum, and the differentiation pad is at its saturation point. Then (step 3) due to the gravity and the larger capillary force in the calibration pad, the calibration pad keeps drawing serum from the differentiation pad, even though the differentiation pad is no longer saturated. Once the calibration pad reaches its saturation point (step 4), it provides no capillary force and stops absorbing serum from the differentiation pad. At this moment, because the differentiation pad has extra absorbing capacity, it holds serum from over-saturating the calibration pad. However, if excessive volume of whole blood is applied to the HYPER pad, the differentiation pad keeps releasing serum at step 4, and thus over-saturates the calibration pad eventually. This leads to a failed quantitative separation.

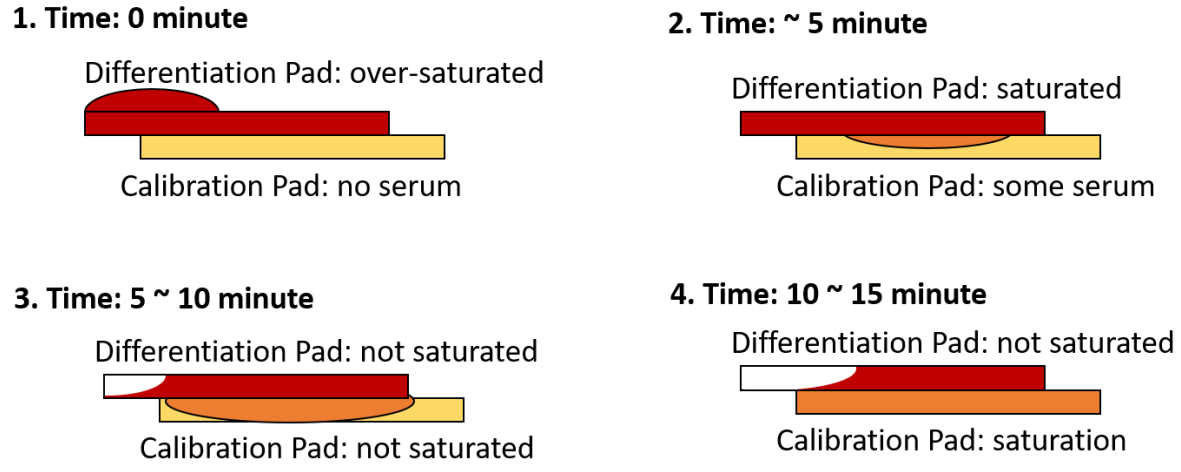

Figure S4. Step-wise illustration on quantitative separation mechanics.

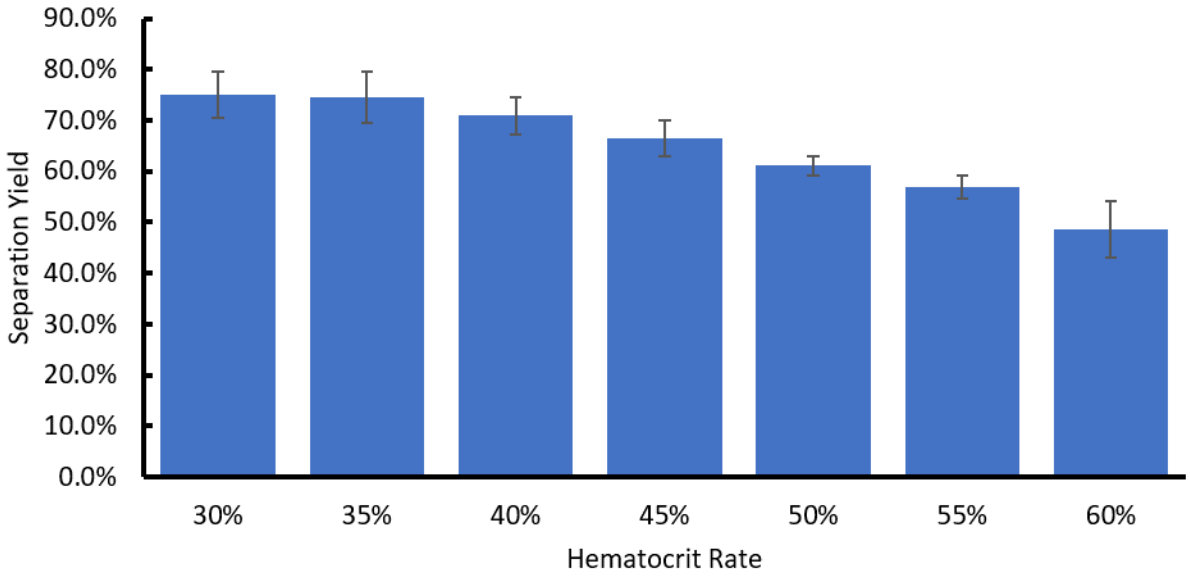

Figure S5. Separation yield versus hematocrit. The plot shows volume of outflow serum as a percentage of total serum in whole blood separated by the HYPER platform at 10 min. 6 tests were performed for each test.

To determine the absorption capacity, we first soak a 1 cm^2 Fusion 5 membrane with 100 µL water. Second, we attach it to another larger piece of Fusion 5 membrane and allow water to freely diffuse between each other. Finally, we measure the weight gain on the 1 cm^2 Fusion 5 membrane piece, which is the water absorption capacity of the Fusion 5 membrane. We obtained a 40.2±0.9 µL/cm^2 water absorption capacity for the membrane, which correspond to the manufacturer's claim that the Fusion 5 membrane has a water absorption at 40 µL/cm^2.

Moreover, to demonstrate the capability of the HYPER platform separation sample in various volume and hematocrit rate, we listed the volume of outflow serum, as a percentage of total serum in whole blood, in Fig. S5. It shows that for 50 ~70 µL whole blood sample, the HYPER pad is able to separate 49% ~ 75% percent of total serum within 10 minutes.

Blood separation efficiency measurement and blood cell count method

The separation efficiency E is defined as the amount of blood cells removed from whole blood sample versus total amount of blood cells. We counted the density of blood cells in the outflow serum N_{out} and the number of blood cells in whole blood sample N_{whole}, and then used them to calculate the separation efficiency of HYPER with equation:

$$E = 1 - \frac{N_{out}}{N_{whole}}$$

To prepare blood cell counting experiment, 5 µL of sample is applied between two pieces of 0.2 mm thick microscope cover slip. Then another piece of cover slip is placed on top of the sample, to form a 0.2 mm thick sample layer. White field microscopy images at 400X magnify were taken for each sample. After image are taken, ImageJ software is used to count the number of blood cells N_{out} and N_{whole}.

In ImageJ software, first we convert the image to grey scale. The image is then converted to binary (black and white) based on a threshold that separates the objects (blood cells) and the background. The number of

cells in the image is counted by the built-in "Analyze Particle" function. The number of cells in the outflow serum and in the whole blood sample were used as N_{out} and N_{whole} to calculate the separation efficiency E. And the results show that the HYPER platform a >99% separation efficiency.

Blood separation result simulation

We utilized the Washburn's equation to predict capillary-driven flow over time. The Washburn's equation, which is a derived from quasi-steady state Poiseuille's equation, is as follows:

$$L^2 = \frac{\gamma D t \cos\phi}{\mu}$$

Where t is the time for fluid, with fluid viscosity μ and surface tension γ, to penetrate a distance of L, in porous media with channel diameter D, and φ is the contact angle between the media and the fluid. Given that flow across intersection of calibration pad is approximately uniform, the volume of blood serum can be estimated by:

$$V = L \cdot A = A \cdot \sqrt{\frac{\gamma D t \cos\phi}{\mu}}$$

Where A is the intersection area of the calibration pad. We then fit the data points with a power function $V = k \cdot t^{1/2}$. We obtained a correlation constant of $R^2 = 0.991$ and a standard deviation of 0.64 μL. Overall, HYPER can obtain 15 μL of serum within 5 minutes, or separate 28.5 μL serum from 60uL whole blood within 20 minutes, giving rise to a high yield at ~86% of available serum.

Supplementary Tables

Table S1: Diagnostics cut-offs for ferritin, RBP and CRP[3]

Biomarker	Concentration in serum	Status
Ferritin	<15 ng/mL (32 pmol/L) for adult <12 ng/mL (22 pmol/L) for children <5 yr < 30 ng/mL (64 pmol/L) with inflammation	Iron deficiency
	> 200 ng/mL (447 pmol/L) for male > 150 ng/mL (335 pmol/L) for female	Iron overload
Retinol-binding protein (RBP)	< 14.7 μg/mL (0.70 μmol/L)	Vitamin A deficiency
	> 22.0 μg/mL (1.05 μmol/L)	Vitamin A sufficient
C-reactive protein (CRP)	< 5 μg/mL (0.15 μmol/L)	No inflammation

Table S2: Fitting parameters for the four parameter logistic curves

Regression Equation	Y=d+(a-d)/(1+(X/c)^b)	
Ferritin		
Parameter	Coefficient	Std. Error
d	7.689e6	1.233e6
a	1.8956	0.3952
c	5.430e8	1.670e8
b	1.0786	0.5091
Sample size	12	
R^2	0.96	
RBP		
Parameter	Coefficient	Std. Error
d	74.655	6.713
a	12.985	0.8702
c	10.737	0.9613
b	-2.7786	3.027
Sample size	12	
R^2	0.81	
CRP		
Parameter	Coefficient	Std. Error
d	34.5342	6.7670
a	2.3719	3.2385
c	4.6004	3.8207
b	0.9778	0.5167
Sample size	12	
R^2	0.98	