

SUPPORTING INFORMATION

EDTA-treated cotton-thread microfluidic device for one-step whole blood plasma separation and assay

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S1 Cotton-thread for μ TAD fabrication

Figure S1 represents cotton thread that is used in the study of cotton-thread microfluidic device for whole blood plasma separation. Cotton thread has specification of 100 % cotton with 32 twists per inch (TPI).

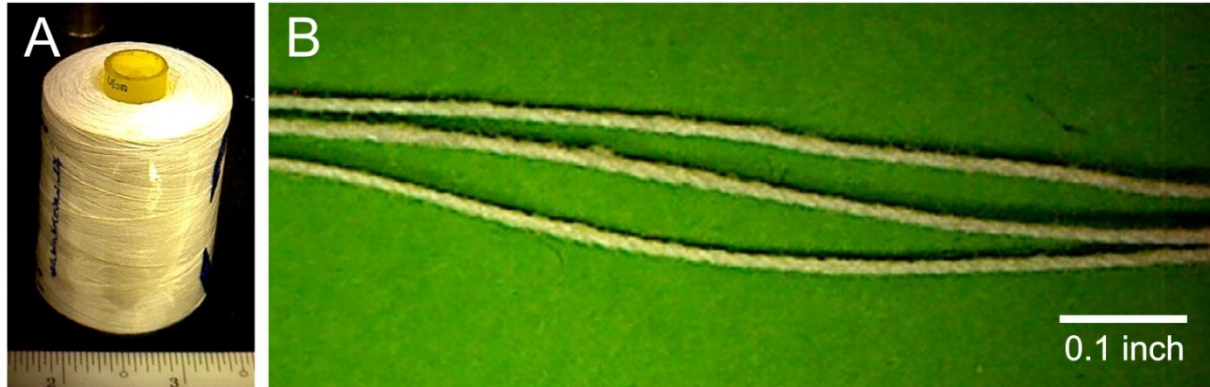


Figure S1: Commercial 100 % cotton thread for blood plasma separation, where (A) yarn and (B) close-up view of the cotton thread.

S2 Evidences of μ TAD after blood separation test

Figure S2 represents the evidences of μ TAD that showing different wicking ability and blood plasma separation. This difference was confirmed by measured the length of wicking and separated plasma among 12 μ TAD types. All tests were conducted at temperature of 30 °C.

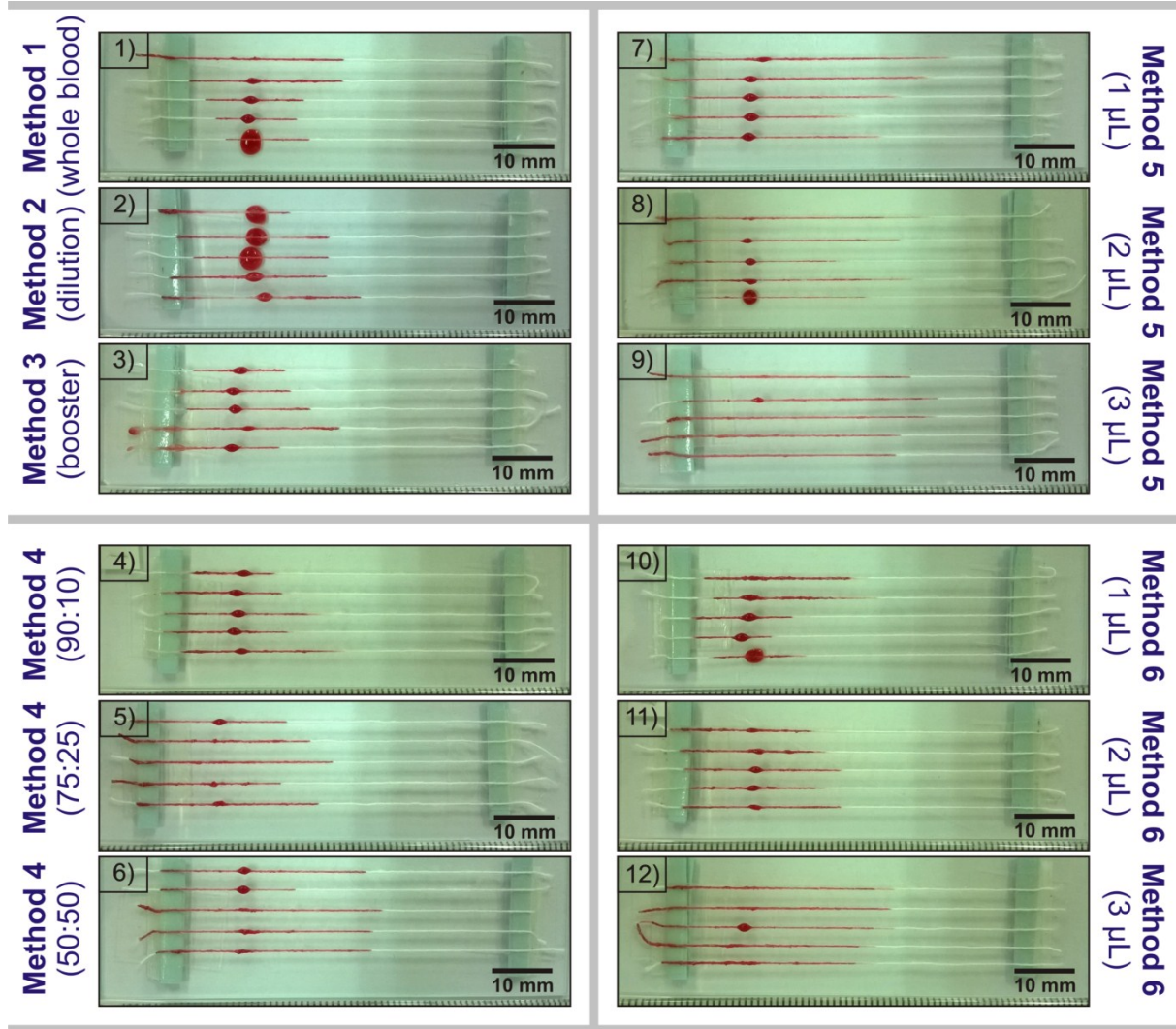


Figure S2: Post-separation blood test at several μ TAD types, where (1) whole blood, (2) dilution, (3) booster, mixed of blood to EDTA in ratio of (4) 90:10, (5) 75:25, (6) 50:50, room-temperature-dried EDTA for 10 s treatments: (7) 1 μ L, (8) 2 μ L, (9) 3 μ L, refrigerated temperature-dried EDTA for 60 min treatments: (10) 1 μ L, (11) 2 μ L, (12) 3 μ L.

S3 Wickability and blood plasma separation analysis

Table S3.1 represents statistical analysis of length of blood wicking and plasma separation among 12 types of μ TADs.

Table S3.1: Statistical analysis of length of blood wicking and plasma separation among 12 types of μ TADs.

Test	Treatments	n	Wicking length [mm]	Plasma length [mm]
(1)	Whole blood	5	22.7 \pm 2.1 ^{i,ii}	n/a
(2)	Dilution			
	1:1	5	26.0 \pm 4.4 ^{i,ii}	n/a
(3)	Booster			
	1:1	5	20.0 \pm 3.6 ⁱ	n/a
(4)	Mixed (Blood : EDTA ratios)			
	90:10	5	28.3 \pm 1.5 ^{ii,iii}	n/a
	75:25	5	34.0 \pm 1.0 ^{iii,iv}	n/a
	50:50	5	40.0 \pm 3.0 ^{iv,v}	n/a
(5)	Room dried at 25 °C for 10 seconds			
	1 μ L EDTA	5	36.3 \pm 0.6 ^{iv}	2.8 \pm 0.3 ^{i,ii}
	2 μ L EDTA	5	42.7 \pm 1.5 ^{v,vi}	2.7 \pm 1.2 ^{i,ii}
	3 μ L EDTA	5	45.3 \pm 2.9 ^{vi}	1.8 \pm 0.3 ⁱ
(6)	Refrigerator dried at 4 °C for 60 min			
	1 μ L EDTA	5	27.0 \pm 1.7 ⁱⁱ	3.0 \pm 1.0 ^{i,ii}
	2 μ L EDTA	5	28.7 \pm 4.2 ^{ii,iii}	3.7 \pm 0.6 ⁱⁱ
	3 μ L EDTA	5	24.7 \pm 4.9 ^{i,ii}	3.0 \pm 0.0 ^{i,ii}

Description: data was displayed in the average \pm standard deviation ($\bar{x} \pm \text{sd}$). The same superscript small roman letters in one column showed no significant differences ($P > 0.05$). n/a = not available.

Table S3.2 represents one-way Analysis of Variance (ANOVA) of the length of blood wicking and plasma separation among 12 types of μ TAD. Results show that wickability of blood were significantly different between 12 types of μ TAD ($P < 0.05$). However, plasma separation was not significantly different between room and refrigerator dried of μ TAD ($P > 0.05$).

Table S3.2: One-way ANOVA test of blood wicking among 12 types of μ TAD and plasma separation between room dried and refrigerator dried of μ TAD

		Sum of Squares	df	Mean Square	F	Sig.
Wickability	Between Groups	2179.556	11	198.141	18.104	.000
	Within Groups	262.667	24	10.944		
	Total	2442.222	35			
Separation	Between Groups	5.333	5	1.067	2.259	.115
	Within Groups	5.667	12	.472		
	Total	11.000	17			

Table S3.3 represent post hoc Duncan of the length of blood wicking among 12 types of μ TAD. Results show that wickability of blood has 6 subset and alpha value at 0.05 among 12 types of μ TAD. This subset further used for superscripts annotation at table S3.1.

Table S3.3: Post hoc Duncan test of wickability analysis among 12 types of μ TAD

Test	Treatment	N	Subset for alpha = 0.05						Superscript (small roman)
			1	2	3	4	5	6	
(3)	Booster 1:1	3	20.0000						i
(1)	Whole blood	3	22.6667	22.6667					i,ii
(12)	Refrigerator 3 μ L	3	24.6667	24.6667					i,ii
(2)	Dilution 1:1	3	26.0000	26.0000					i,ii
(10)	Refrigerator 1 μ L	3		27.0000					ii
(4)	Mixed 90:10	3		28.3333	28.3333				ii,iii
(11)	Refrigerator 2 μ L	3		28.6667	28.6667				ii,iii
(5)	Mixed 75:25	3			34.0000	34.0000			iii,iv
(7)	Room 1 μ L	3				36.3333			iv
(6)	Mixed 50:50	3				39.0000	39.0000		iv,v
(8)	Room 2 μ L	3					42.6667	42.6667	v,vi
(9)	Room 3 μ L	3						45.3333	vi
Sig.			.051	.060	.057	.092	.187	.333	
Means for groups in homogeneous subsets are displayed.									

Table S3.4 represent post hoc Duncan of the length of plasma separation between room dried and refrigerator dried of μ TAD. Results show that plasma separation has only 2 subset and alpha value 0.05 between room dried and refrigerator dried of μ TAD. This subset was used for superscripts annotation at table S3.1.

Table S3.4: Post hoc Duncan test of plasma separation analysis between room dried and refrigerator dried of μ TAD

Test	Treatment	N	Subset for alpha = 0.05		Superscript (small roman)
			1	2	
(9)	Room 3 μ L	3	1.8333		i
(8)	Room 2 μ L	3	2.6667	2.6667	i,ii
(7)	Room 1 μ L	3	2.8333	2.8333	i,ii
(10)	Refrigerator 1 μ L	3	3.0000	3.0000	i,ii
(12)	Refrigerator 3 μ L	3	3.0000	3.0000	i,ii
(11)	Refrigerator 2 μ L	3		3.6667	ii
Sig.			.082	.129	
Means for groups in homogeneous subsets are displayed.					

S4 Time-lapse of μ TAD after blood separations test

Figure S4 depicts time-lapse of blood wicking and plasma separation among 12 types of μ TAD that was recorded up to 120 s.

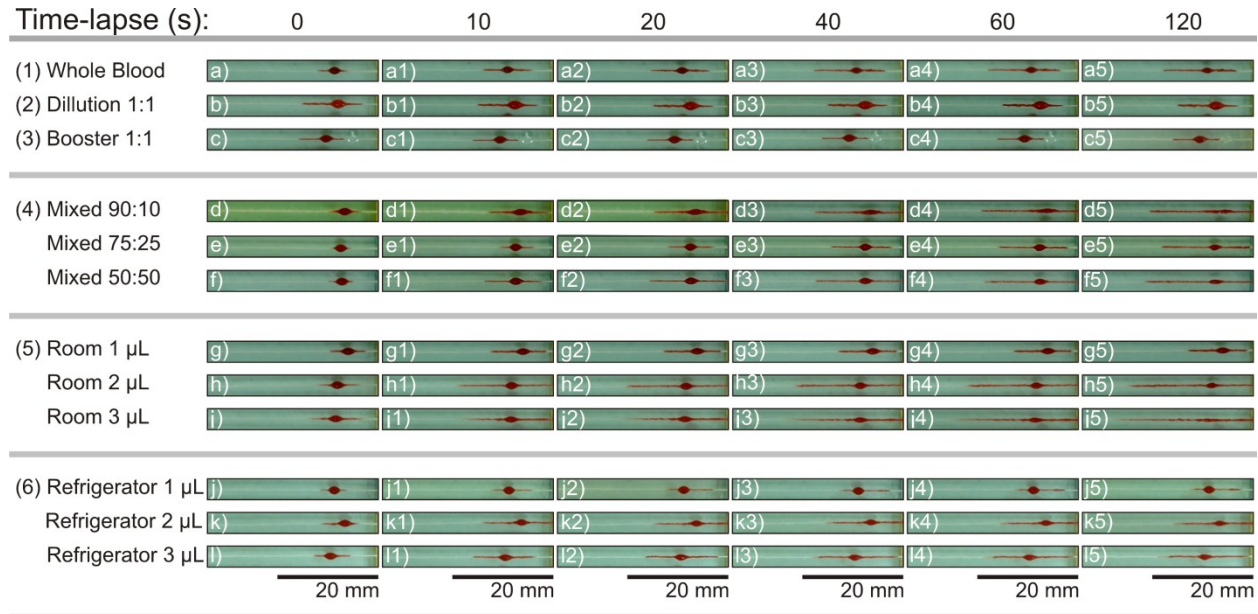


Figure S4: Time-lapse of blood test among 12 types of μ TAD, where (a,a1-5) whole blood, (b,b1-5) dilution, (c,c1-5) booster, mixed of blood to EDTA in ratio of (d,d1-5) 90:10, (e,e1-5) 75:25, (f,f1-5) 50:50, room-temperature-dried EDTA for 10 s treatments: (g,g1-5) 1 μ L, (h,h1-5) 2 μ L, (i,i1-5) 3 μ L, refrigerated temperature-dried EDTA for 60 min treatments: (j,j1-5) 1 μ L, (k,k1-5) 2 μ L, (l,l1-5) 3 μ L.

S5 Cell analysis of μ TAD after blood separations test

Figure S5 represents giemsa staining of wet blood smear that depicts amount of the blood cells among 12 types of μ TAD.

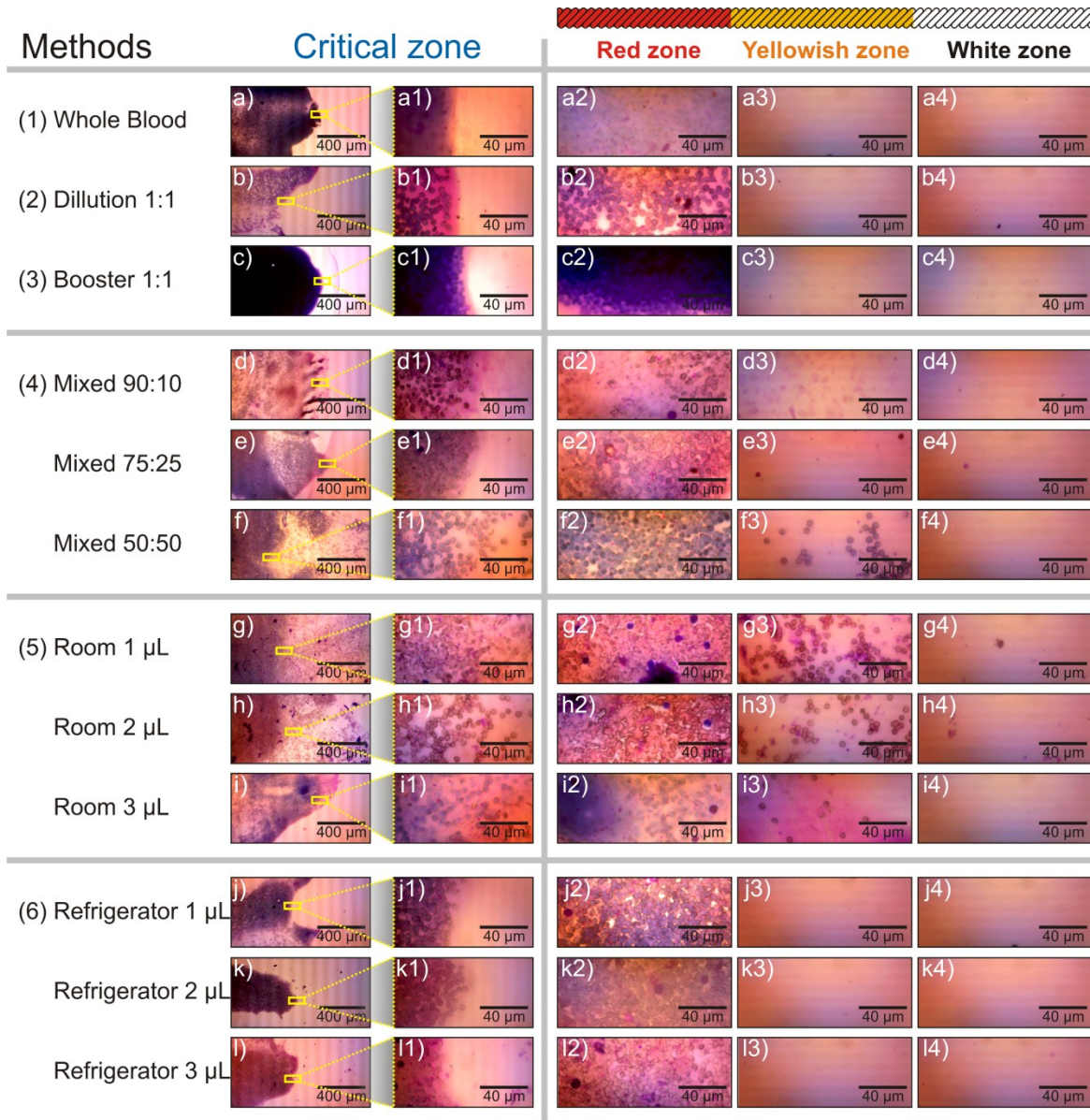


Figure S5: Cell analysis on several μ TAD types, where (a,a1-4) whole blood, (b,b1-4) dilution, (c,c1-4) booster, mixed of blood to EDTA in ratio of (d,d1-4) 90:10, (e,e1-4) 75:25, (f,f1-4) 50:50, room-temperature-dried EDTA for 10 s treatments: (g,g1-4) 1 μ L, (h,h1-4) 2 μ L, (i,i1-4) 3 μ L, refrigerated temperature-dried EDTA for 60 min treatments: (j,j1-4) 1 μ L, (k,k1-4) 2 μ L, (l,l1-4) 3 μ L.

S6 Sheep's blood haematocrit and total protein profile

Figure S6 represents quantification measurement of haematocrit and total protein in sheep blood that used in this study.

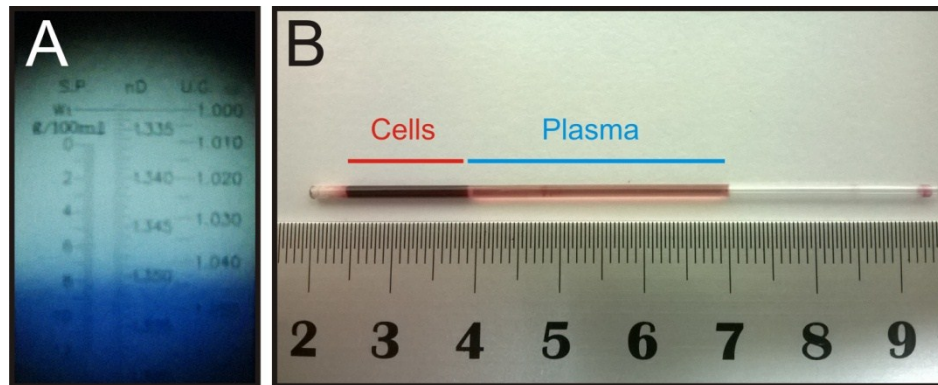


Figure S6: Total protein, specific gravity and haematocrit measurement of blood sheep, where (A) image of hand-refractometer from centrifuged blood plasma and (B) image of centrifuged blood in the capillary microhaematocrit tube for haematocrit calculation.

Sheep blood profile:

1. Haematocrit

$$\begin{aligned}
 \text{Plasma high} &= 3.1 \text{ cm} \\
 \text{High blood sediment (Ts)} &= 1.4 \text{ cm} \\
 \text{Total high (Th)} &= 4.5 \text{ cm} \\
 \text{Haematocrit} &= (Ts/Th) \times 100 \% \dots\dots\dots (1) \\
 &= (1.4/4.5) \times 100 \% = 31.11 \%
 \end{aligned}$$

$$2. \text{ Specific gravity} = 1.350$$

$$3. \text{ Total protein (g/dL)} = 8 \text{ g/dL}$$

4. Albumin

$$\begin{aligned}
 \text{Albumin (g/dL)} &= 60 \% \times \text{Total protein} \dots\dots\dots (2) \\
 &= 0.6 \times 8 = 4.8 \text{ g/dL}
 \end{aligned}$$

S7 Effect of voluminous to the efficacy of μ TAD

Figure S7 represents effect of various volume deposition of EDTA-treat vs. blood on the μ TAD that showing in the difference length (mm) on blood wicking and plasma separation.

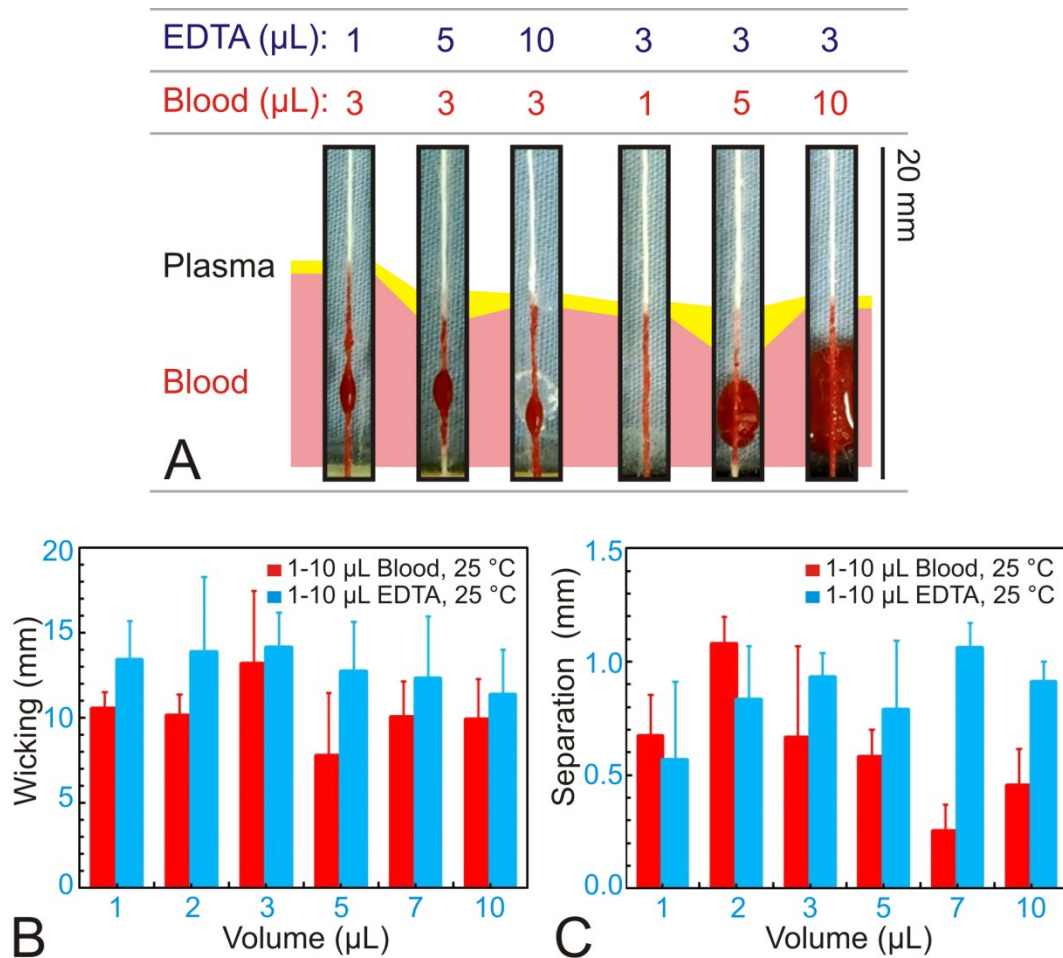


Figure S7: Variation of liquids volumes (EDTA-treatment vs. blood) on the blood wicking and plasma separation of μ TAD. (A) Selected images from the testing with illustration of blood wicking and plasma separation that tested at 25 °C after 120 s, (B) length of blood wicking (mm), and (C) length of plasma separation.

S8 Effect environmental temperature to the efficacy of μ TAD

Figure S8 represents effect of environmental temperature to the blood wicking and plasma separation of μ TAD. Effect of environmental temperature was studied by using 1-3 μ L of EDTA-treatment on cotton-thread with 3 μ L blood sample for 120 s.

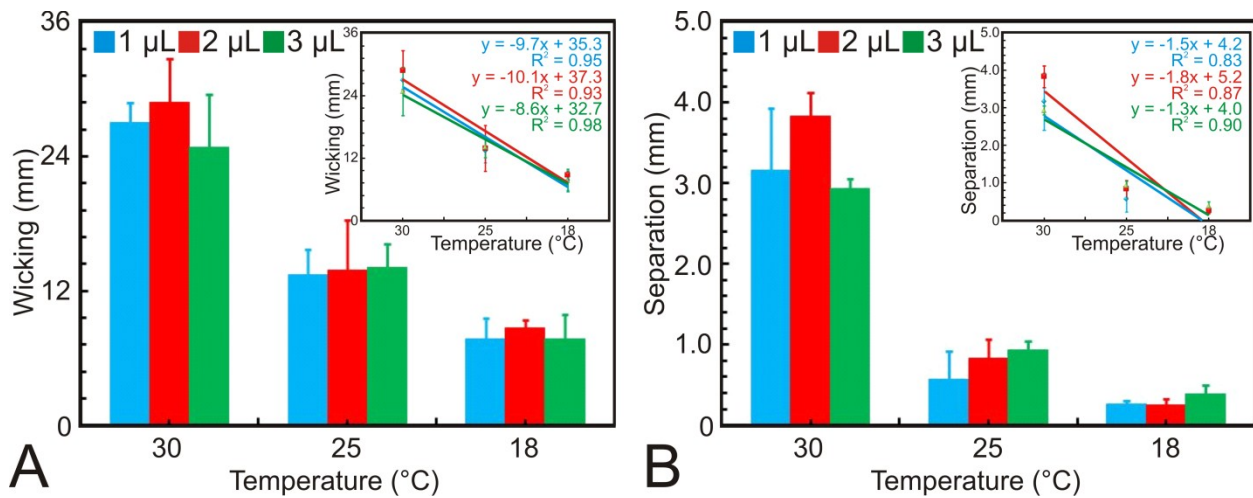


Figure S8: Effect of environmental temperature to the blood wicking and plasma separation of μ TAD on the (A) length of blood wicking and (B) plasma separation.

S9 Comparison with several finding on blood separation methods

Table S9 shows comparison of several methods and findings on the blood plasma separation.

Table S9: Comparison methods and findings on blood plasma separation with our study

No	Parameters	Ref S10.1	Ref S10.2	Ref S10.3	This study
1.	Material				
	Length	~50 mm	15 mm	~20 mm	20 mm
	Specification	Nitrocellulose paper	Filter paper	Polyester thread	Cotton thread
2.	Pre-treatment				
	Type	No need	Wax-impregnated	Plasma-treatment	Scouring
	Method	No data	Soldering iron-impregnated	Vacuum plasma	Na ₂ CO ₃
	Time	No data	>30 min	60 s	10min; boiled
3.	Agent				
	Type	CaCl ₂	NaCl / MgCl ₂	Anti-ABO and anti-Rh/D antibodies	Ethylene diamine tetra acetic acid
	Concentrations	0.1-0.5 M	0.154-0.68 M	No data	10 %
	Method	Mixed with sample	Dropping	Soaking	Dropping
	Volume	15 µL	0.5 µL	~>1000 µL	1-3 µL
	Preparation	No need	3 min; room temperature	10 min; fume hood	60 min; refrigerator
4.	Blood				
	Treatment	Dilute blood (160 µL blood + 5 µL NaCl + 15 µL CaCl ₂)	On-paper dilution (immobilized salt-solution)	No need	No need
5.	Working test				
	Volume	100 µL	5 µL	0.6-4 µL	1-3 µL
	Time	240 s	60 s	No data	30-120 s
	Separation	4-8 mm	2-3 mm	3 mm	3 mm
6.	Assay/Test				
	Type	Blood clotting	Glucose	Blood typing	Albumin
	Analysis	Colorimetric	Colorimetric	Colorimetric	Colorimetric
	Agent	CaCl ₂	2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)	Anti-ABO and anti-Rh/D antibodies	Bromcresol green
	Volume	Dilute sample (160 µL blood + 5 µL NaCl + 15 µL CaCl ₂)	1 µL	Soaking (~>1000 µL)	1-3 µL
	Preparation time	No data	6 min; room temperature	6 min; room temperature	60 min; refrigerator temperature
	Working time	240 s	20 min	No data	30 s
	Separation	4-8 mm	No data	No data	No data
7.	Expiry				
		No data	No data	4 week; refrigerator in micro-tubes wrapped in foil	7 day; room temperature without packaging
Note: Not good; Enough; Very good.					

S10 Comparison efficiency on blood plasma separation

Table S10 represents efficiency of methods and findings compared with our study. The efficiency of the separation was calculated by formula:

$$\text{Separated plasma (\%)} = (\text{separation} / \text{wicking}) \times 100 \% \quad (3)$$

$$\text{Entrapped plasma (\%)} = \text{plasma} - \text{separated plasma} \quad (4)$$

$$\text{Efficiency (\%)} = (\text{separated plasma} / \text{plasma}) \times 100 \% \quad (5)$$

Table S10: Efficiency of methods and findings compared with our study

No	Parameters	Ref S10.1	Ref S10.2	Ref S10.3	This study
1	Blood source	Rabbit	Human	Human	Sheep
2	Haematocrit (%)	35	40	40	32
3	Plasma (%)	65	60	60	68
4	Blood volume (μL)	100	5	4	3
5	Wicking (mm)	13.0-15.5	8.0-9.0	9.0-9.5	25.0-30.0
6	Separation (mm)	8.0	1.0-2.0	3.0-3.5	3.0-3.8
7	Separated plasma (%)	52-62	13-22	33-37	12-13
8	Entrapped plasma (%)	3-13	38-47	23-27	55-56
9	Efficiency (%)	80-96	22-37	55-62	17-19
Note: Ref 1-3, haematocrit (%) and plasma (%) value was calculated from the normal value of rabbit and human. Furthermore, the length (mm) of wicking and separation was measured from the image presented on the manuscript.					

References S10:

S10.1. H. Li, D. Han, G. M. Pauletti and A. J. Steckl, *Lab Chip*, 2014, **14**, 4035-4041

S10.2. A. Nilghaz and W. Shen, *RSC Advances*, 2015, **5**, 53172-53179.

S10.3. D. Ballerini, X. Li and W. Shen, *Analytical and Bioanalytical Chemistry*, 2011, **399**, 1869-1875.

S11 Comparison of calculated cost of used reagents

Table S11.1 comparison of calculated cost in powder form of used reagents.

	Ref S11.1	Ref S11.2	Ref S11.2	This study
Reagent	CaCl ₂	NaCl	MgCl ₂	EDTA
Specification	Anhydrous, powder, ≥ 97 %	BioXtra, ≥ 99.5 % (AT)	Anhydrous, ≥ 98 %	BioUltra, Anhydrous, ≥ 99 %
Company brand	Sigma-Aldrich	Sigma-Aldrich	Sigma	Sigma-Aldrich
CAS number	10043-52-4	7647-14-5	7786-30-3	60-00-4
Form	Powder	Powder	Powder	Powder
Packaging (g)	100	250	100	100
Price/pack (SGD)	122.50	78.20	103.00	48.70
Price/g (SGD)	1.225	0.313	1.030	0.487
Note: *accessed from http://www.sigmaaldrich.com/ (date: 02/02/2016)				

Table S11.2 comparison of calculated cost in solution form of used reagents.

	Ref S11.1	Ref S11.2	Ref S11.2	This study
Reagent	CaCl ₂	NaCl	MgCl ₂	EDTA
Specification	BioUltra, for molecular biology	BioUltra, for molecular biology	for molecular biology	BioUltra, for molecular biology, pH 8.0
Company brand	Sigma	Sigma	Sigma	Sigma-Aldrich
Concentration (M)	1	5	1	0.5
CAS number	10043-52-4	7647-14-5	7786-30-3	60-00-4
Form	Solution	Solution	Solution	Solution
Packaging (mL)	1	1000	100	100
Price (SGD)	74.30	169.50	89.10	74.90
Price (SGD)				
Price/M	74.30	33.90	89.10	149.80
Price/M/mL	74.30	0.034	0.891	1.498
In device application				
Concentration (M)	0.5	1	0.67	0.1
Immobilized (μL)	15	0.5	0.5	1
Cost/application (SGD)	557.250	0.017	0.298	0.150
Note: *accessed from http://www.sigmaaldrich.com/ (date: 02/02/2016)				

References S11:

S11.1. H. Li, D. Han, G. M. Pauletti and A. J. Steckl, *Lab Chip*, 2014, **14**, 4035-4041.

S11.2. A. Nilghaz and W. Shen, *RSC Advances*, 2015, **5**, 53172-53179.

S12 Miniaturize platform and comparison of albumin assay

Figure S12 represents miniaturized length of μ TAD. Regarding to previous results on 50 mm thread length platform, therein shows that only 30-35 mm length the blood wick on-thread. Furthermore, the μ TAD platforms were then miniaturized from 50 mm into 30 mm length. A more small volume of fluids, 1 μ L EDTA, 1 μ L albumin (BSA) and 2 μ L distilled water or BCG were used in albumin detection in artificial blood plasma.

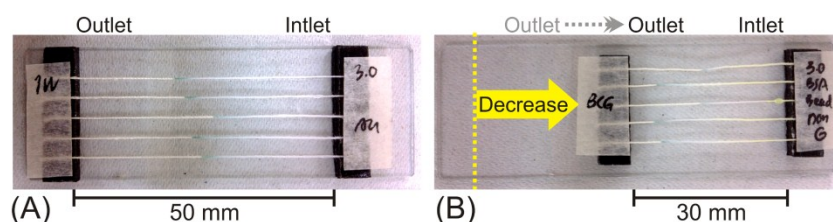


Figure S12: Miniaturize length of μ TAD from (A) 50 mm into (B) 30 mm.

Table S12.1: Comparison of albumin detection using bromocresol green (BCG) kits

[illegible]

S13 Comparison among several commercial products

Table S13 represents comparison between several commercial blood analyzer tools.

Table S13: Comparison among several commercial products

Comparison	Comercial products				This study
	i-STAT 1* Abbott, New York	Element POC Heska, Colorado***	VetScan** Abaxis, Canada	Element DC Heska, Colorado	µTAD
Unit type	Portable	Portable	Stationer	Stationer	Portable
Parameter(s)	12	11	10	12	1
Biochemistry	iCa, Na ⁺ , K ⁺ , Cl ⁻ , L, BUN, UREA, Glu, Creat, pH, PCO ₂ , PO ₂ , No ALB	iCa, Na ⁺ , K ⁺ , Cl ⁻ , L, Glu, Creat, Hct, pH, PCO ₂ , PO ₂ , No ALB	ALB, ALP, AST, Ca ²⁺ , CK, GGT, Mg, PHOS, TP, BUN	ALP, ALT, BUN, CREA, Glu, TP, TBIL, ALB, PHOS, Ca ²⁺ , CHOL, GGT	ALB
Volume	300-450 µL****	~100 µL	90-120 µL	50 µL	1-3 µL
Vol/parameter	17-95 µL	9 µL	9-12 µL	4 µL	1-3 µL
Preparation	No need	No need	600 s	60-360 s	No need
Calibration	In device-calibration	165 s (card)	In device-calibration	No data	In device - calibration
Working	130-1000 s	35 s	840 s	300 s	120 s
Total time	130-1000 s	200 s	1440 s	360-660 s	120 s
System	potentiometry, hydrolyzed, conductometrically, amperometrically,	potentiometry, conductometrically, amperometrically,	colorimetry	colorimetry, potentiometry	colorimetry
Energy	AC 100-240 Volts DC 2x9 Volts Lithium-ion Batteries	AC 100-240 Volts DC Lithium-ion Batteries	AC 100-240 Volts	AC 100-240 Volts	No external energy
System	Cartridge-based system	Card-based system	Rotor-based liquid embed reagents	E-wrap panels system (embed drying reagent)	Microfluidic cotton-embed drying reagent
Type	19 different cartridge	1 card	10 different rotor	1 panel	1 glass slide
Storage	2-8 °C (<i>long-time</i>) 18-30 °C (<i>fast-time</i>)	18-30 °C	2-8 °C	18-30 °C	25-30 °C
Expiry	5 min (25 °C)-individual cartridge 1 h (25 °C)-cartridges box	No data	20 min (25 °C)-open pack 48 h (25 °C)-closed pack	No data	168 h-no packaging
Note: *) https://www.abbottpointofcare.com/products-services/istat-test-cartridges/menu ; i-STAT Manual ART: 714446-00S; **) Abaxis VetScan® leflet (PN: 500-7113 Rev: C); ***) https://www.heska.com/Products/Lab-Systems.aspx ; ****) 2-3 drops; 1 drop = 150µL; Albumin (ALB), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Calcium (Ca ²⁺), Creatine kinase (CK), Gamma glutamyl transferase (GGT), Magnesium (Mg), Inorganic phosphorus (PHOS), Total protein (TP), Urea nitrogen (BUN), Sodium (Na), Potassium (K), Chloride (Cl), Glucose (GLU), Creatinine (CREAT), Ionized Calcium (iCa), pH, Carbon dioxide (PCO ₂), Oxygen (PO ₂), TCO ₂ , Lactate (L), Haematocrite (Hct), Haemoglobine (Hb); Total bilirubin (TBIL); Cholesterol (CHOL); Not good; Enough; Very good.					

S14 Comparison of LoD and LoQ between separation and centrifugation

Table S14 presents comparison of Limit of Detection (LoD) and Limit of Quantification (LoQ) of semi-quantitative albumin detection in artificial blood using μ TAD by separation and centrifugation methods. The LoD value of separation and centrifugation were 0.0114 g/dL and 0.0133 g/dL, respectively. Moreover, the LoQ value of separation and centrifugation were 0.0381 g/dL and 0.0442 g/dL, respectively.

Table S14: Comparison of Limit of Detection (LoD) and Limit of Quantification (LoQ).

Artificial blood	Separation		Centrifugation	
Curve equation ($Y_1, X_1; \dots; Y_5 X_5$)	$y = 0.6484 \ln(x) + 0.002$		$y = 0.6265 \ln(x) - 0.0128$	
R square	0.991		0.995	
Slope	0.6484		0.6265	
Original colour intensity data				
Value of $Y_1; X_1$	Y_1 (a.u.)	$X_1 = e^{(y-0.002)/0.6484}$	Y_1 (a.u.)	$X_1 = e^{(y+0.0128)/0.6265}$
1	0.000556	0.977976	0.010625	1.038098
2	0.000734	0.980669	0.009306	1.035915
3	0.000631	0.979102	0.006597	1.031445
4	0.000347	0.974829	0.008958	1.035340
Mean of $Y_1; X_1$	0.000567	0.978144	0.008872	1.035199
Standard deviation (SD_1)	0.000164	0.002471	0.001678	0.002771
LoD calculation				
LoD	$= (3 \times SD_{X_1})/\text{slope}$ $= (3 \times 0.002471) / 0.6484$ $= 0.0114 \text{ g/dL} \sim 0.01 \text{ g/dL}$		$= (3 \times SD_{X_1})/\text{slope}$ $= (3 \times 0.002771) / 0.6265$ $= 0.0133 \text{ g/dL} \sim 0.01 \text{ g/dL}$	
LoQ calculation				
LoQ	$= (10 \times SD_{X_1})/\text{slope}$ $= (10 \times 0.002471) / 0.6484$ $= 0.0381 \text{ g/dL} \sim 0.04 \text{ g/dL}$		$= (10 \times SD_{X_1})/\text{slope}$ $= (10 \times 0.002771) / 0.6265$ $= 0.0442 \text{ g/dL} \sim 0.04 \text{ g/dL}$	

S15 Comparison of mechanism and separated products

Table S15 comparison of several mechanisms on blood plasma separation using simple and low-cost device

No	Sampling	Sample processing steps	Mechanism of separation	Separated product	Ref.
1	Conventional plasma: Blood + anticoagulant (e.g. EDTA, citrate, heparine, hirudin)	(1) whole blood centrifugation, (2) separation	Mechanical force: Anticoagulant prevent blood to clot and centrifugal force separated plasma	Pure plasma	S15.1
2	Conventional serum: Blood + without anticoagulant (e.g. plain) or using coagulant (e.g. CaCl_2)	(1) clotted blood centrifugation, (2) separation	Mechanical force: Coagulant induce blood to clot and centrifugal force separated serum	Pure serum	S15.1
3	LFA device: Blood + anticoagulant (citrate)	(1) diluted blood, (2) total blood clot, (3) filtration NaCl solution + blood + CaCl_2 solution	Hard osmotic-induce: Excess Ca from CaCl_2 salt in hypertonic solution induce <u>totally diluted blood to clotting</u> and nitrocellulose paper used as filter to separated plasma-serum in lateral flow capillarity action	Diluted plasma-serum (water-contaminant) + Na and Ca (salt-contaminant)	S15.2
4	μ PAD: Blood + anticoagulant (Li-heparin)	(1) diluted blood, (2) cells shrivel, (3) partial clot, (4) separation NaCl solution + blood, MgCl_2 solution + blood	Soft osmotic-induce: Excess Na or Mg from NaCl or MgCl_2 salt, respectively induce <u>partially of blood cells shrivelled, coagulation and entrapped the</u> blood cells in hypertonic solution at functionalized-Whatman filter paper to separated plasma-serum by lateral flow capillarity action	Diluted plasma-serum (water-contaminant) + Na or Mg (salt-contaminant) + intracellular fluid (fluid cell-contaminant)	S15.3
5	μ TAD : Blood + anticoagulant (citrate)	(1) diluted blood, (2) twist per inch sorting, (3) separation	Twist per inch: Excess anticoagulant boosting blood wick and separating blood cells and plasma	Diluted plasma-serum (anticoagulant-contaminant)	S15.4
6	μ TAD : Blood + anticoagulant (EDTA.K3)	(1) EDTA dissolve (2) fibrin-filter from less part of blood clot at downstream blood wicking (cotton-fiber), (3) separation EDTA-dried + blood + cotton-fiber	Fibrin-filter: EDTA treatment (dried) delayed blood to clot that induced by surface charge of cotton-fiber, especially at the downstream wicking blood (free EDTA thread part). Fibrin snare formed and functionalized as filter, namely fibrin-filter.	Plasma-serum	This study

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