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

Supporting information

Rapid and easy-identifiable blood typing on microfluidic cotton thread-based analytical devices

Shuqiang Min‡[a], Tonghuan Zhan‡[a], Yang Lu[b], Deng Pan[c], Xiaoqing Chen[b]* and Bing Xu[a]*

a School of Mechanical Engineering, Suzhou University of Science and Technology, Suzhou, 215009, China

b Department of spinal surgery, Affiliated hospital of Nantong University, Nantong, 226001, China

c Information Materials and Intelligent Sensing Laboratory of Anhui Province, Anhui University, Hefei, 230601 China

E-mail: xq.c@live.com & xb022@ustc.edu.cn

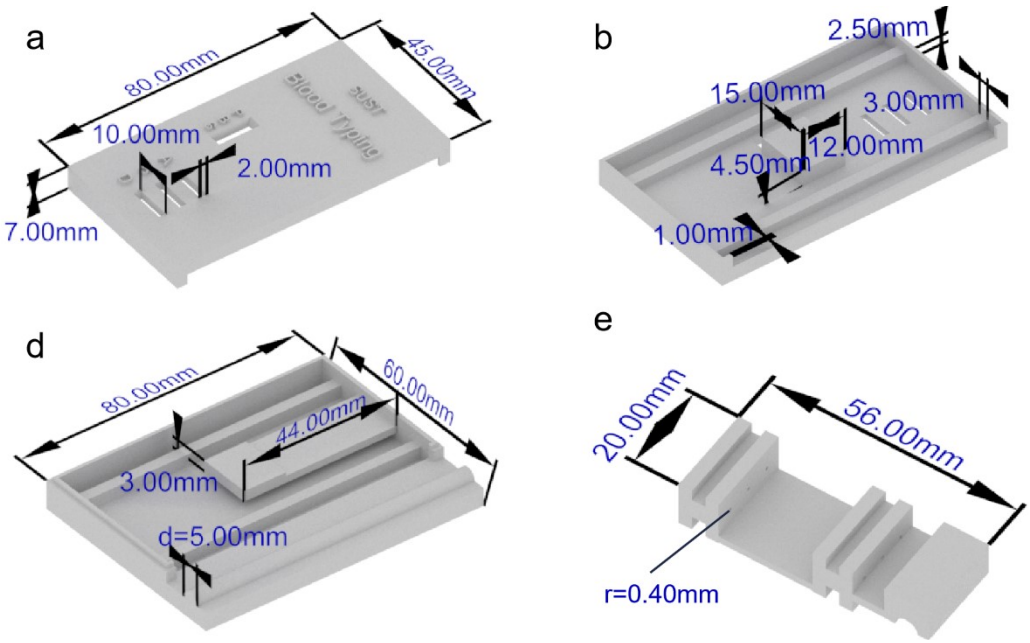


Figure S1. The size parameters of blood-typing chip. a) The front side of upper cover. b) The reverse side of the upper cover. c) The lower substrate. d) The slider with three holes for fixing cotton threads.

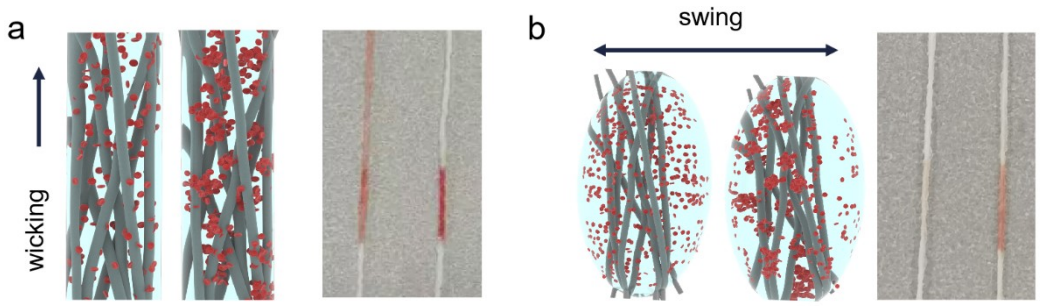


Figure S2. The mechanism diagrams between traditional wicking-elution method and swing-elution method. a) In wicking-elution method, PBS buffer is wicked on the cotton thread, which can help to flow free RBCs to a long distance. However, a large number of free RBCs can still be trapped inside the interfiber gaps. In addition, free RBCs can only travel in the wicking direction, and there is no other place to flow. As a result, there is still a clear blood stain on the negative assay (no agglutination reaction), meanwhile the positive assay has a similar result. Unfortunately, the wicking-elution method produces an ambiguously result, which cannot be easily and precisely identified by nonprofessional users. b) In swing-elution method, the threads move back and forth (in more directions). Initially, free RBCs can be trapped on the threads, but they can be still eluted away due to the repetitive swing movements and the stronger elution force. Besides, the free RBCs can be released to the external PBS buffer, resulting in no or invisible blood stains on the cotton threads. Therefore, the swing-elution method can provide much more clear blood-typing results, which can be easily detected by the naked eye, even to nonprofessional users.

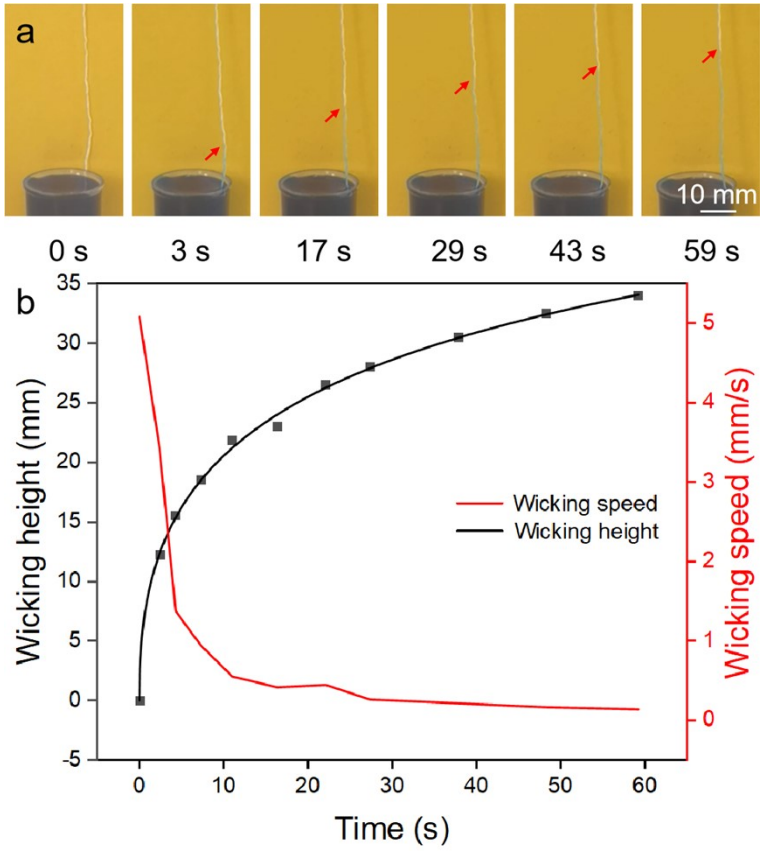


Figure S3. The wicking property of a single cotton thread. a) The actual wicking process of dyed liquid on a cotton thread. b) The relationship between the wicking height/speed and the flow time.

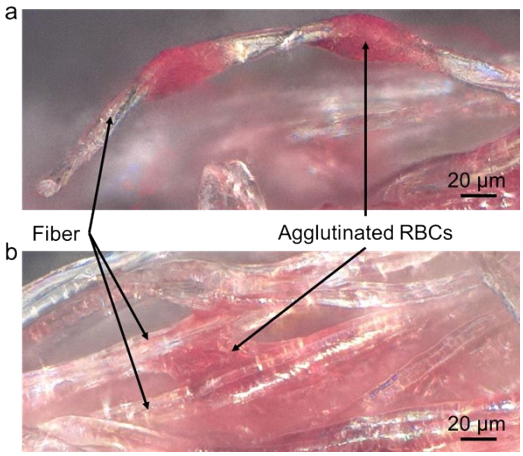


Figure S4. The images of agglutinated RBCs attached to fibers and embedded in fiber structure after positive assay through a super depth of field 3D microscope system. After positive assay, the thread shows a clear red color due to a large number of large agglutinated RBCs embedded in the fibrous structure.

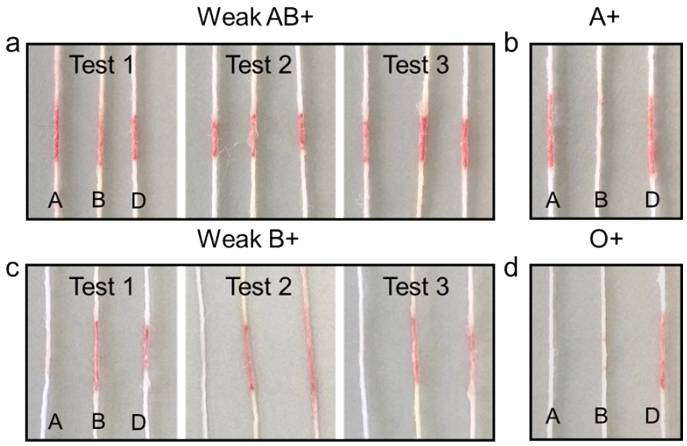


Figure S5. The actual detection results of a) weak AB+ (weak B subgroup), b) A+, c) weak B+ (weak B subgroup) and d) O+ blood types through the swing-elution method. To ensure the accuracy of the experiments, we conduct three tests on the same blood sample (AB+ and B+, weak B subgroup). The experiments show that the three test results are consistent for weak AB+ and B+ (all three anti-B coated threads had obvious red blood stains). For comparison, the normal A+ and O+ blood types are also detected by our method and there are no obvious red blood stains on the anti-B coated threads (although several free RBCs still remains). From our results, it is relatively easy to identify weak AB+/B+ blood types and normal types.

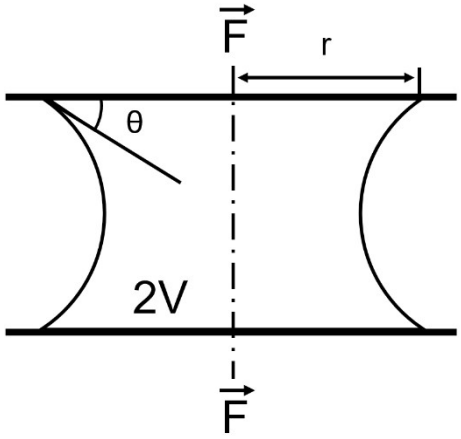


Figure S6. Schematic diagram of liquid bridge between parallel plates. Δp is the pressure difference across the curved interface, the r is the radius of contact line of the fluid interface with the plates, γ is the interfacial tension and θ is the angle of contact of the fluid with the plates. Fa represents cohesion force.

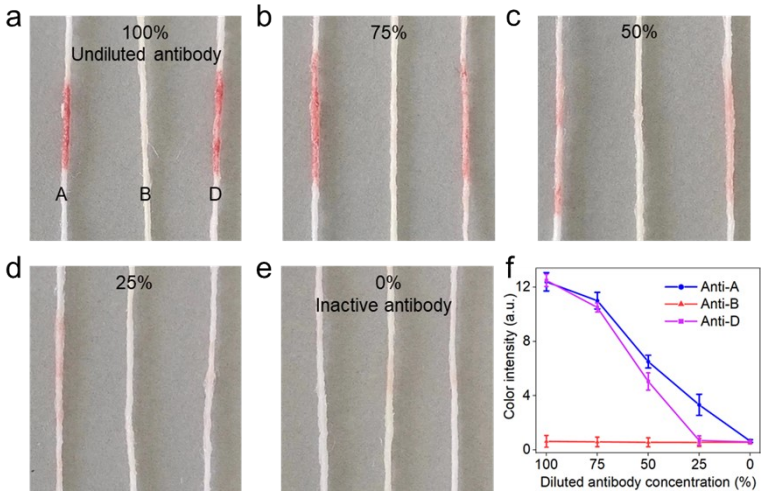


Figure S7. a-e) The test results of using different diluted antibody concentration of 100% (undiluted), 75%, 50%, 25% and 0% (inactive antibody). f) Analysis of the color intensity of blood spots after swing-elution. Error bars give the standard deviation of the color intensity of each thread from 3 test samples. Here we mainly test the A+ blood type. The results show that our swing-based elution method is sensitive to an undiluted antibody (100%) and diluted antibodies with a concentration of 75%/50% by the observation of our naked eyes. Overall, the concentration of antibodies has a great impact on our method. When the antibody is diluted, the sensitivity of the test decreases.

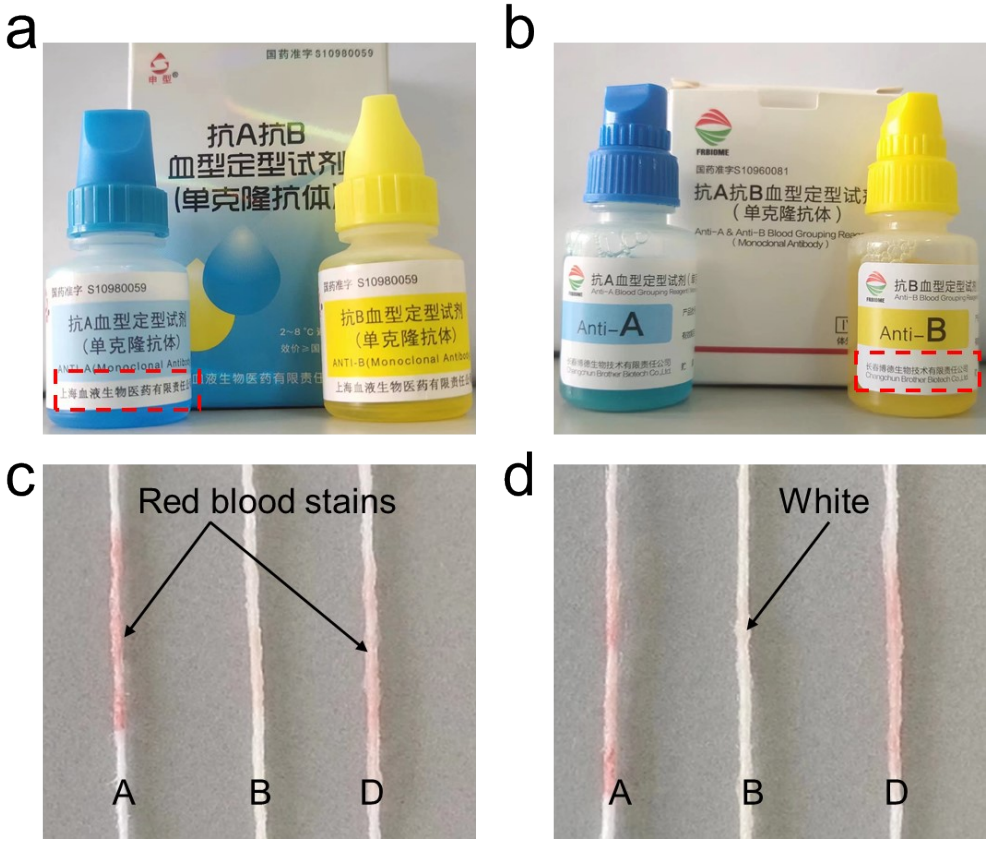


Figure S8. The optical images of monoclonal antibodies which were obtained from different companies, a) Shanghai Blood Biopharmaceutical Co., Ltd (Shanghai, China) and b) Changchun Bode Biotechnology Co., Ltd (Changchun, China). c) and d) The blood typing results using different antibodies (all diluted by 50%). Although we do not know the specific concentration of the antibodies purchased, the testing results of using different antibodies obtained from different companies are consistent. For A+ blood type, there are obvious red blood stains on the left and right threads, while the middle threads show white color.

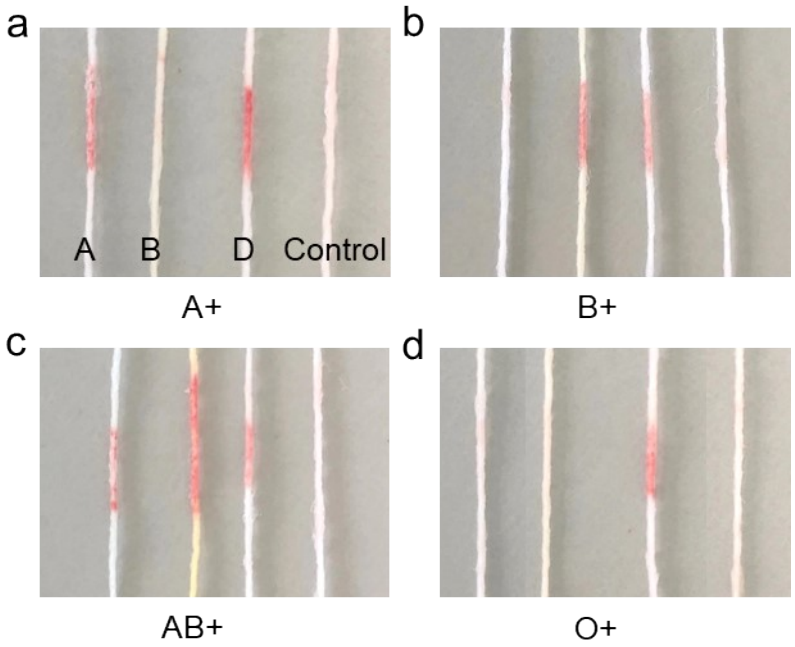


Figure S9. a-d) The results of blood typing with a control group. The thread on the far right is the control group (only adding phosphate buffers). If a red blood stain appears on the control thread, the result is invalid, which indicates that the blood sample can self-coagulate at room temperature. The results show that the blood samples used have no self-coagulation phenomenon.

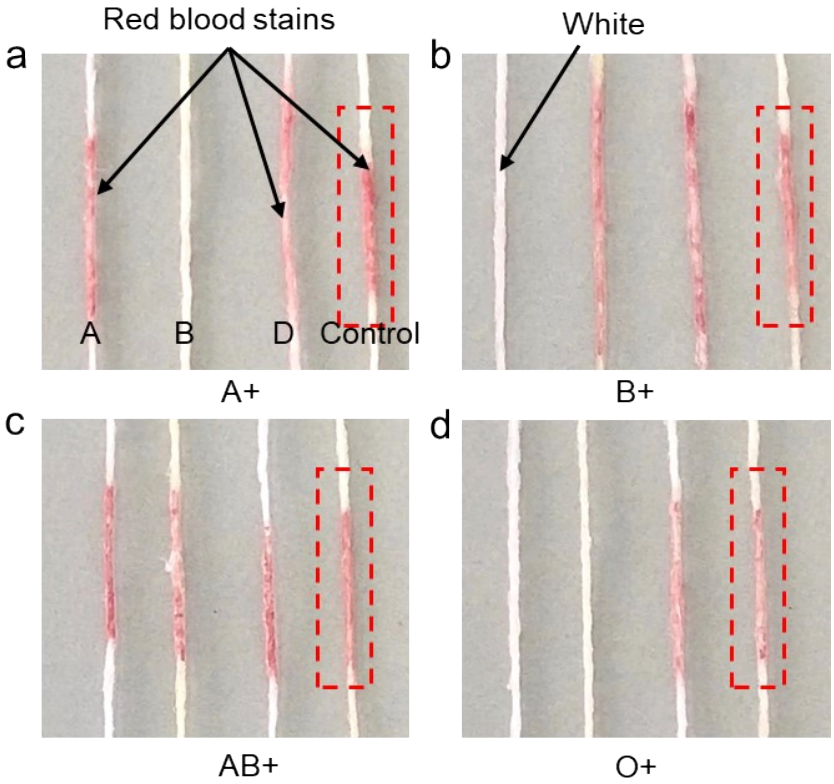


Figure S10. a-d) The results of blood typing with a control group (adding Anti-A, -B and -D, each antibody: 3 μL). In these experiments, the control group is expected to show obvious red stains and prove that the antibodies are active. For B+ blood type, the threads coated with Anti-B and Anti-D will show obvious red blood stains, while that coated with Anti-A do not. If the control group shows a white color, there is no antibody that reacts with the test blood sample, indicating that the antibodies are inactive. In our experiments, the antibodies that we use are active.

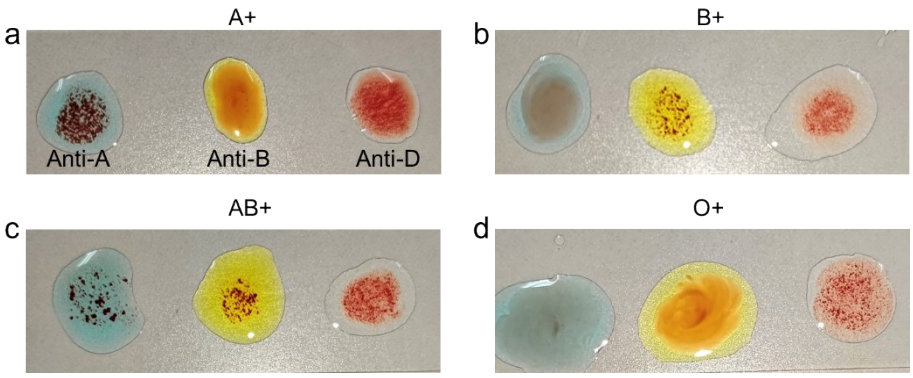


Figure S11. a-d) The results of the plate-type blood typing using the antibodies stored at 4 ℃ for several months. The results indicate that the antibodies after long-term conservation are still active and can function correctly.

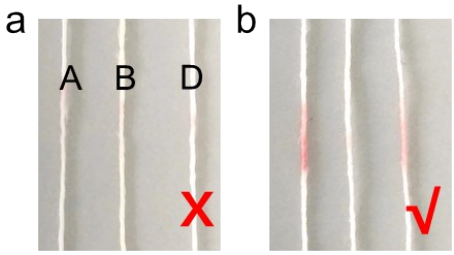


Figure S12. The test results of threads with antibodies stored at different conservation ways for 7 days a) at room temperature in summer and b) at 4 ℃. The red ticks indicate that our method is valid, while the red crosses indicate that our method is invalid. Low-temperature storage of antibodies can maintain the activity of the antibodies, thereby not affecting the detection results (e.g., for A+ blood type, there are obvious red blood stains on the left and right threads, while the middle threads show white color.).

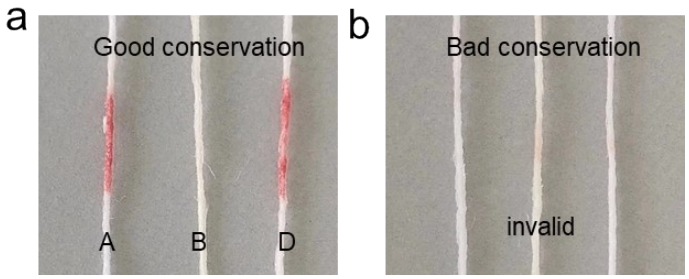


Figure S13. The test results of using antibodies stored at different conservation ways, a) at 4 ℃ and b) at room temperature. Antibodies stored under low-temperature conditions can maintain their activity well, allowing for accurate detection of blood types. For comparison, storing antibodies incorrectly can cause antibodies to become inactive, resulting in incorrect test results.

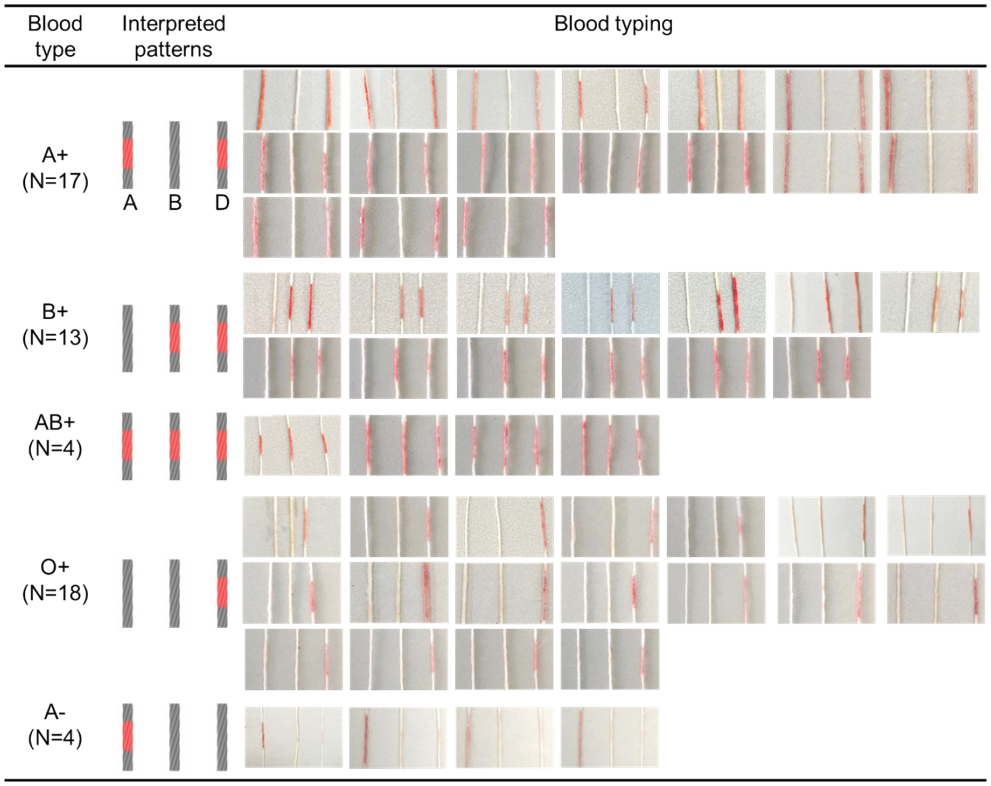


Table S1. Blood group typing by swing-elution. Relying on our method, the detection accuracy is 100%.

Reference	Fabrication difficulty	Is a portable device prepared?	Testing procedure	Blood samples required	Elution solution required	Cost
Lab chip, 2015, 24, 4533-4541	Difficulty (CNC machining + plasma modification +bonding **+polymer**)	Yes	3 steps (1. add antibodies 2. add blood samples 3. press the screw to provide pump force)	~1 μL	0 μL	High
ACS Sens. 2020, 5, 3082–3090	Difficulty (CNC machining + plasma modification +bonding **+polymer**)	Yes	4 steps (1. add antibodies 2. add PBS solution 3. add blood samples **4. Pull the rod of the syringe pump to create negative pressure)**	~1 μL	~10 μL	High
J Immunoass Immunoch, 2018, 39, 1486856	Difficulty (wax printing **+paper**)	Yes	4 steps (1. add antibodies 2. add blood samples **3. add modified LISS** 4. wash to elute)	~2×25 μL	~2×300 μL	High
Anal. Chim. Acta, 2016, 921, 67-76	Difficulty (wax printing **+paper**)	No	4 steps (1. add antibodies×2 2. add blood samples **3. add elution solution to dilute blood** 4. add diluted blood)	~10 μL	~30 μL	High
Analyst, 2021,146, 1048-1056	Easy (**paper**)	No	3 steps (1. add antibodies 2. add blood samples 3. take photos to analyze)	~10 μL	0 μL	Middle
ACS Appl. Mater. Interfaces, 2014, 6, 22209−22215	Easy (**silk thread**)	No	3 steps (1. add antibodies 2. add blood samples 3. put threads into the eluent tank)	~3×0.5 μL	~100 μL	Middle
Anal. Bioanal. Chem， 2011, 399, 1869–1875	Difficulty (plasma modification **+polyester thread**)	No	3 steps (**1. add water-soluble dyes to the clear anti-D solution** 2. add antibodies 3. add blood samples	~1 μL	0 μL	High
This work	Easy (**cotton thread**)	Yes	3 steps (1. add antibodies 2. add blood samples 3. swing threads in eluent)	~3×0.5 μL	~200 μL	Low

Table S2. Comparison of different blood typing methods, of which our portable thread-based blood typing devices have the advantages of simple fabrication, low cost, easy to use, and minimal sample consumption.

Reference	Lab chip, 2015, 24, 4533-4541	ACS Sens. 2020, 5, 3082–3090	J Immunoass Immunoch, 2018, 39, 1486856	Anal. Chim. Acta, 2016, 921, 67-76	Analyst, 2021,146, 1048-1056	ACS Appl. Mater. Interfaces, 2014, 6, 22209−22215	This work
Antibody solution required	~3×0.5 μL	~3×0.5 μL	~2×3 μL	~3×3 μL	~30 μL	~4×2 μL	~3×3 μL
Price/assay	_	_	0.03	0.03	>0.03	0.02	0.01

[a]This price only considers antibody consumption, elution solution and materials.
[b] The 30 μL of antibody solution costs $0.03 in Analyst, 2021,146, 1048-1056.

Table S3. The volume of antibody solution required and the price of per assay from different works. The price only includes the cost of antibody solutions, elution solution, and materials. For better comparison, it is assumed that the antibody solution and eluent used are the same. The materials are Whatman filter paper [J Immunoass Immunoch, 2018, 39, 1486856; Anal. Chim. Acta, 2016, 921, 67-76], paper tower [Analyst, 2021,146, 1048-1056], silk [ACS Appl. Mater. Interfaces, 2014, 6, 22209−22215], and cotton thread (our work).

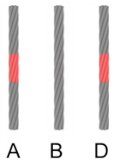
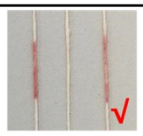
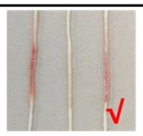
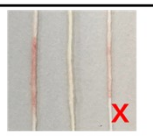
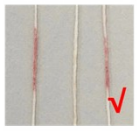
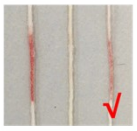
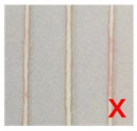
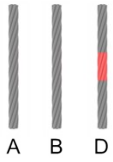
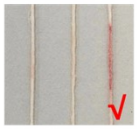
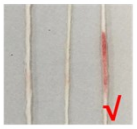
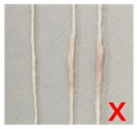
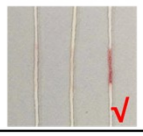
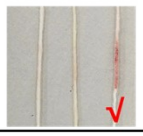
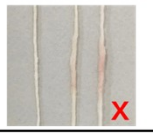
Blood group	Sample	Interpreted pattern	Ambient condition		
			Natural humidity, indoor 20 ℃	Natural humidity, outdoor 38 ℃	High humidity, indoor 20 ℃
A+	Sample 1				
	Sample 2				
O+	Sample 3				
	Sample 4				

Table S4. The effect of different environment parameters on the test results. In our experiments, the high humidity is ~97% and the natural humidity is ~48%. The tick in the figure indicates that our method is valid under the set environmental conditions, while the cross indicates that our method is invalid.