3D microfluidic analytical device on a single thread for smart point-of-care blood typing

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# Equal contribution.

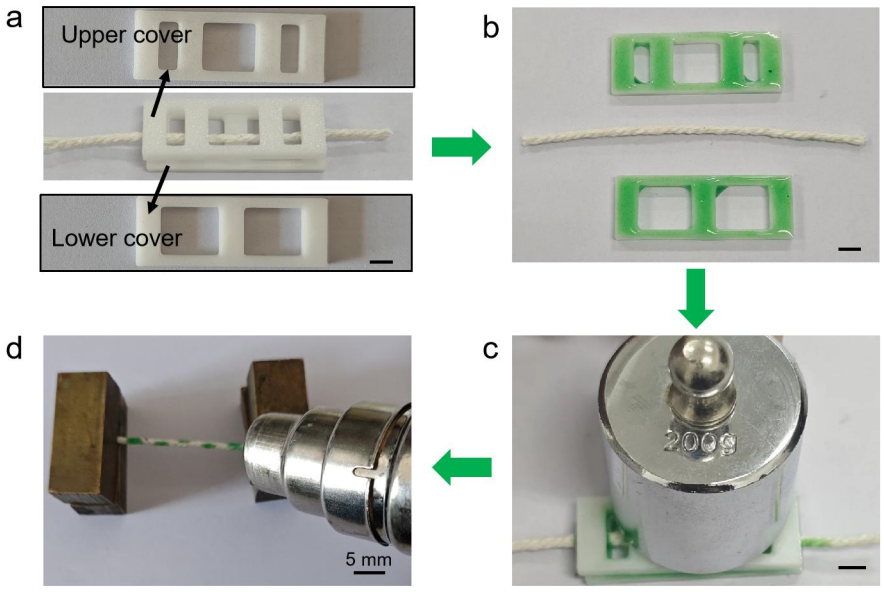


Fig. S1. Fabrication process of the 3D-μSTAD. a) The mask templates involve the upper and lower covers with several different windows. b) The templates were firstly coated with hydrophobic PDMS, which would formed a uniform thin film on their surface due to tension. c) Then, the templates were carefully aligned and pressed onto the thread, with the press of standard quality weight. d) Finally, the hot gun were utilized to accelerate the welding process of PDMS.

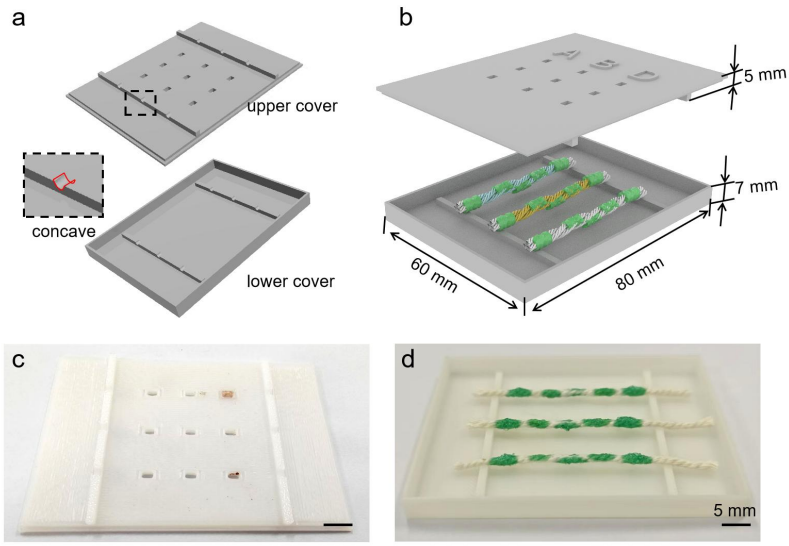


Fig. S2. a) The detailed construction of the upper and a lower cover in 3D-μThread-BT chip. On both cover, there were several concave structures that enable the 3D-μSTADs to be fixed and suspended in the air. b) The shells of 3D-μThread-BT chip consist of an upper cover and a lower cover. And the upper cover features several windows designed for introducing samples and observing the final test results. c) and d) The actual images of the upper and lower covers.

```python
def correct_brightness_and_color(img):

    hsv_img = cv2.cvtColor(img, cv2.COLOR_BGR2HSV)
    h, s, v = cv2.split(hsv_img)

    t = 200

    def correct_channel(channel):

        average_c = np.mean(channel)

        diff_c = max(0, t - average_c)

        correct_c = channel + diff_c

        correct_c = np.clip(correct_c, a_min: 0, a_max: 255).astype(np.uint8)
        return correct_c

    correct_v = correct_channel(v)


    brightness_correct_img = cv2.cvtColor(cv2.merge((h, s, correct_v)), cv2.COLOR_HSV2BGR)


    return brightness_correct_img
```

Fig. S3. The pseudocode for image's RGB calibrate algorithm.

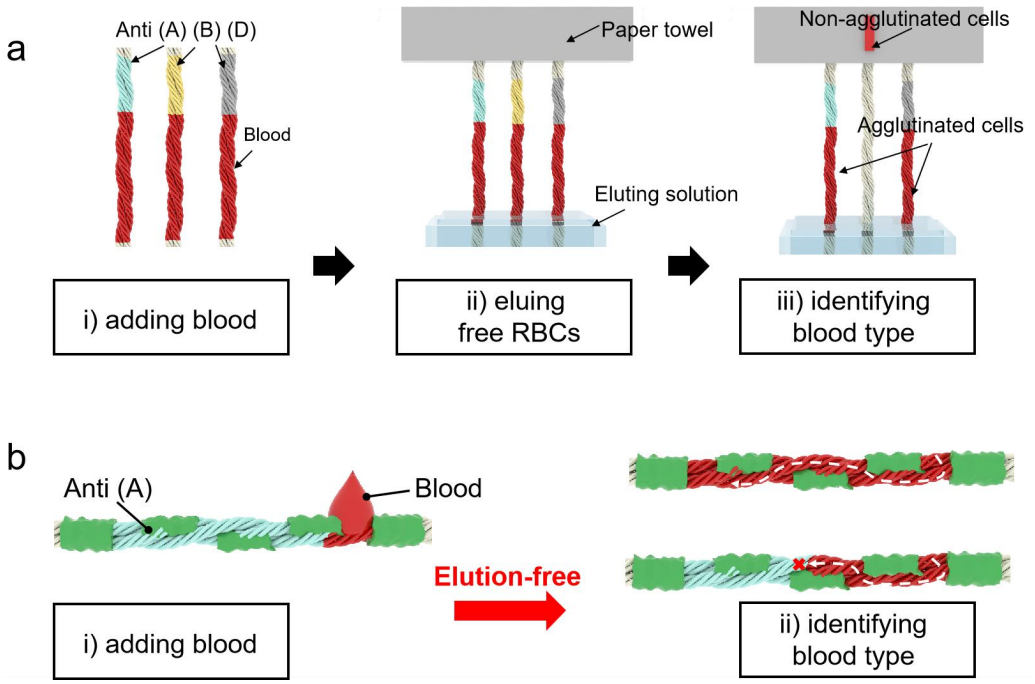


Fig. S4. a) Traditional blood typing process in 2D μTADs, the elution is always required to elute free RBCs while retain agglutinated RBCs [ACS Appl. Mater. Interfaces, 2014, 6(24), 22209-22215]. b) Our 3D-μSTADs eliminate the requirement of elution step.

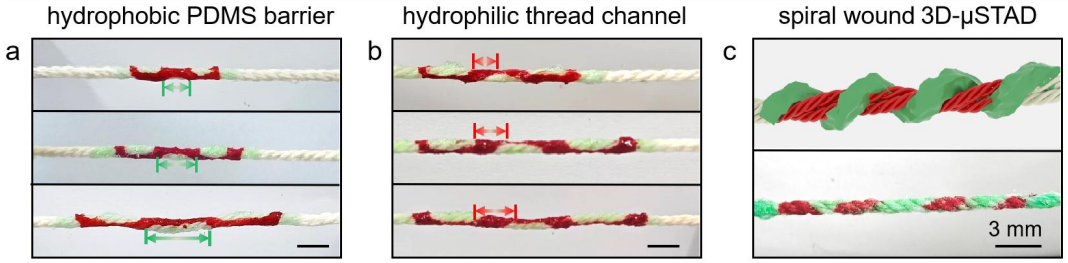


Fig. S5. Different shapes of the 3D-μSTADs. a) By altering the lengths of hydrophobic PDMS barrier, the corresponding length of hydrophilic channels can be adjusted. b) By modifying the hydrophilic thread channel, the distance traveled by the blood flow can be varied. c) By using a flexible thread filled with PDMS, and winding spirally around the cotton thread, we can create a spiral channel within 3D-μSTADs. Herein, the green barrier was coated with the PDMS, while red channel was dyed by red blood.

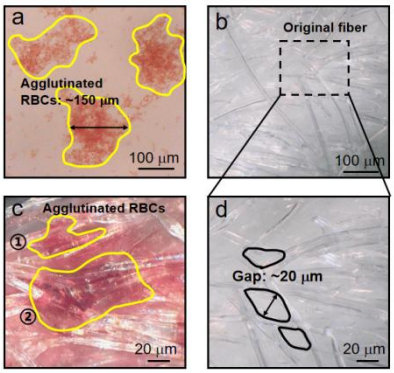


Fig. S6. a) The images of the agglutinated RBCs on the glass slide, showing that their size are in hundreds micrometers. b) and d) The super depth microscope images of the original cotton fiber, showing the gaps are in tens of micrometers. c) The small and large agglutinated RBCs are firmly captured in the gaps.

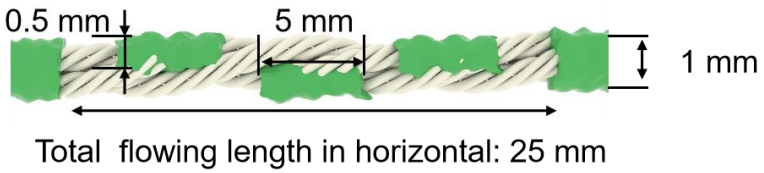


Fig. S7. The specific geometric parameters of the proposed 3D-μSTADs. Here, the horizontal length of the flowing path is set to 25 mm, and the thickness of hydrophobic PDMS barrier is 0.5 mm with a length of 5 mm, and the diameter of hydrophilic cotton thread is 1 mm.

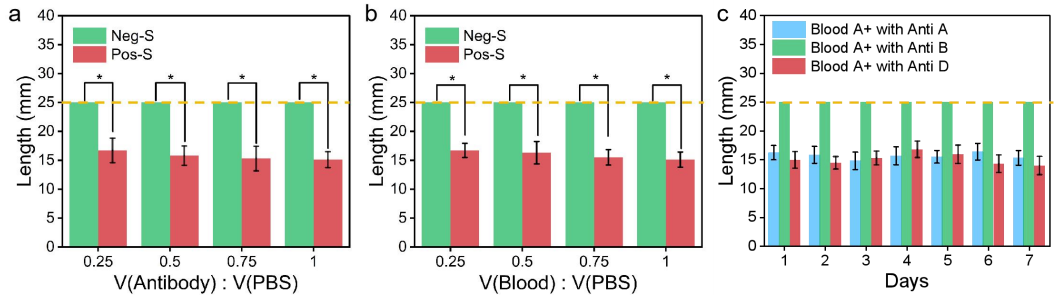


Fig. S8. The detection length of different volume radio. a) The relationship between the diluted antibodies and the length of Neg-S and Pos-S (n=5). The results indicate that even the antibody is diluted to 1/4 concentration, there still remains a distinct difference in the length of the two results. b) The relationship between the diluted Blood sample and the length of Neg-S and Pos-S (n=5). Even the blood is diluted into 1/4, the detection results can also be recognized. Both of these findings demonstrated the reliability and stability of our method. c) The relationship between the storage duration of the blood sample and the flow length is investigated. The results demonstrate the compatibility of our methods with stored blood samples (n=5).

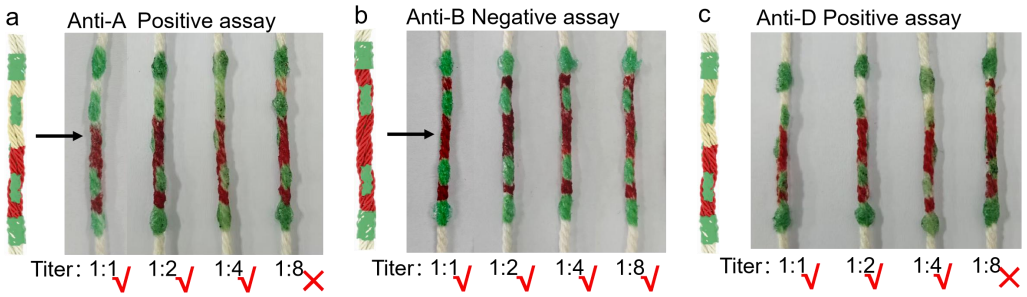


Fig. R9. The actual detection results at different antibodies. The A+ blood sample is used and the antibody is diluted by the PBS buffer. a) and c) In the positive assay where anti-A and anti-D are coated in the 3D-μSTADs, the blood sample cannot be accurately classified in antibodies with a titer of 1:8. This can be attributed to the fact that high dilution ratio corresponds to a lower antibody concentration, potentially leading to inadequate antigen-antibody reactions that fail to promptly obstruct blood flow in the positive assay. b) In the negative assay, the blood flow remains uninterrupted regardless of the antibody concentration since the antigen-antibody reaction would not occur. And the titer of the antibodies can be concluded into 1:4.

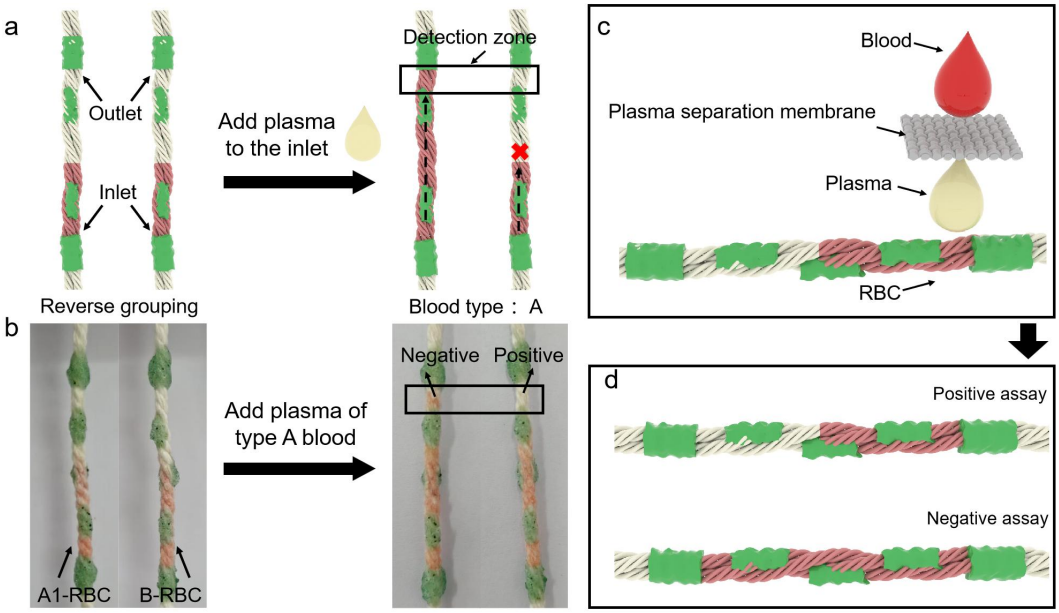


Fig. S10. a) The schematic diagram of reverse grouping in 3D-μSTADs, with A1 and B RBC (12 μL) pre-coated on the front half of the thread. The blood typing result can be observed at the outlet (detection zone) after adding the plasma (30 μL) in the inlet. b) Actual images of the reverse typing in 3D-μSTADs, in negative assay, the plasma continues to flow towards the outlet, leaving a significant red stain. While in positive assay, the plasma is halted by the agglutinated RBCs resulting from the hemagglutination reaction, leaving the outlet white. c) and d) The concept of our future work in combining the plasma separation membrane to achieve an integrated reverse testing device.

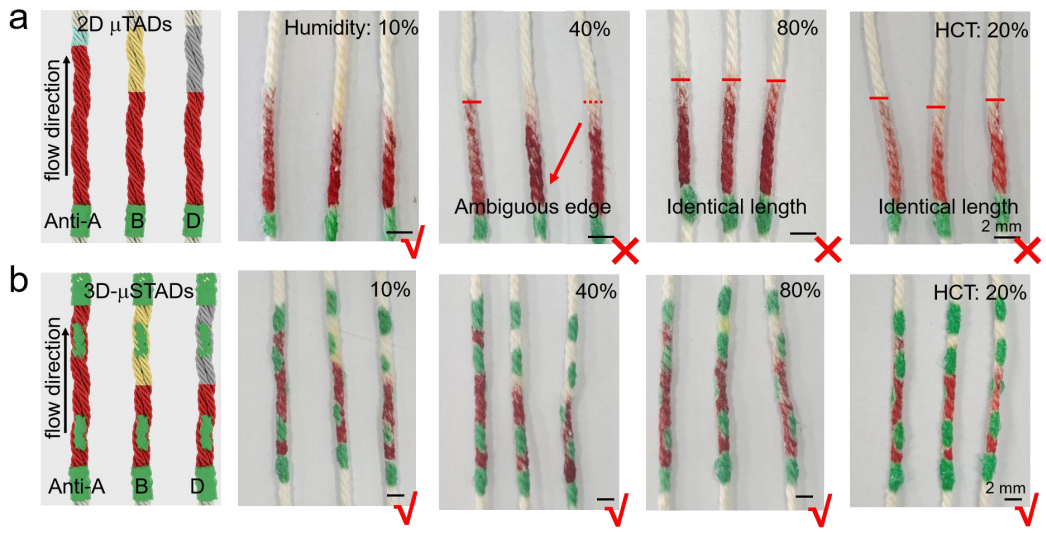


Fig. S11. The comparison between 2D μTADs with 3D-μSTADs. The 2D μTADs was fabricated by directly precoating the antibody solutions (22.5 μL, equal to the blood volume) on the thread. The blood type (B+, HCT=40%, 22.5 μL) can be identified according to the blood flow length. a) For the 2D μTADs, the blood typing results are unreliable in high humidity condition or low HCT sample. b) Attributing to the complex fibrous structure of 3D-μSTADs, our system are capable of detecting blood type in high humidity environment or low HCT sample.

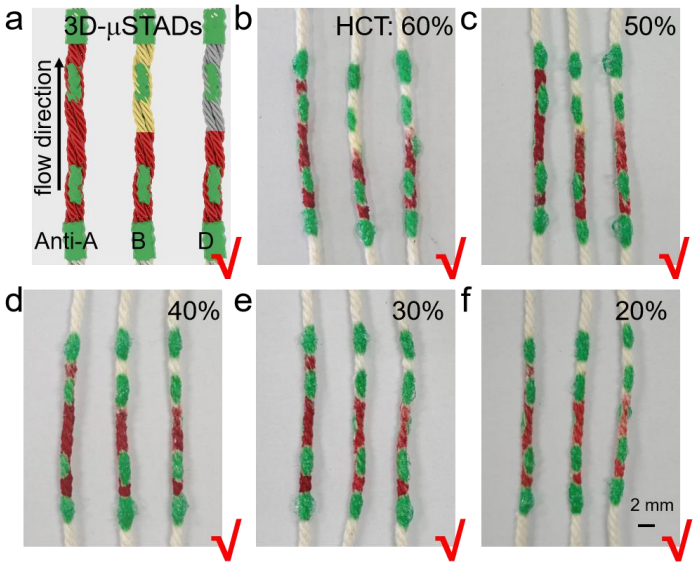


Fig. S12. a) The diagrammatic sketch of blood typing result (B+) in 3D-μSTADs. b-f) The actual blood typing results of blood sample with different HCT.

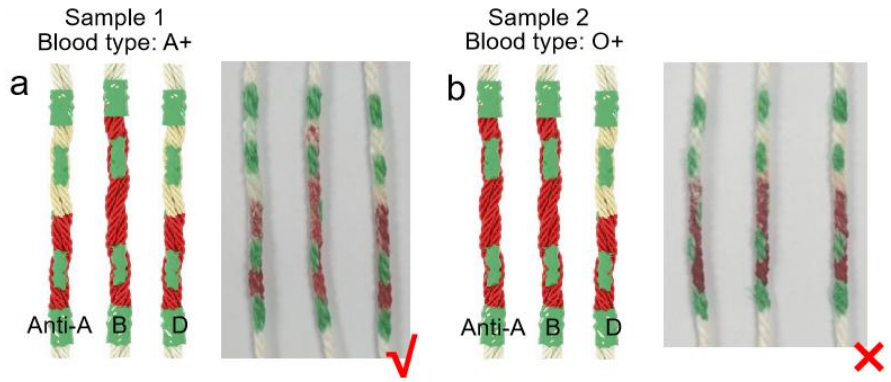
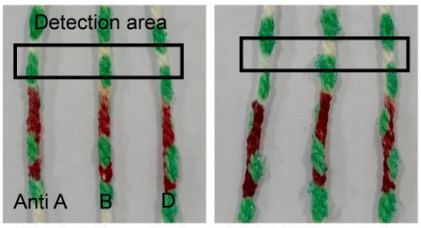


Fig. S13. a) For the DAT-positive sample 1 (A+), the 3D-μSTADs can successfully classify the blood type. b) For the DAT-positive sample 2 (O+), all the blood flow were halted in the middle, indicating the false classification of 3D-μSTADs.

a



(A_3B+) Sample 1 (A_3B+) Sample 2

b

Blood type	RBCs to be tested +reagent antibody					serum to be tested+reagent RBCs				
	-A	-B	-AB	-A	-H	A1	A2	B	O	Self cells
A_3B	3+mf	4+	4+	0/w mf	0/w	0/w	0	0	0	0

1. mf: In the test tube method, it shows a mixed field of vision (two groups of cells separated up and down appear in the gel test), and A3 and B3 agglutination cells are about 1/2.

2. 'w' indicates weak agglutination, usually less than 2+; The "0 (el)" test tube centrifugation method is negative, and the absorption and release test can detect the corresponding antigen.

3. The parentheses in "[?]" indicate the presence of cold reactive antibodies; Any "X/" format (such as "w/0") indicates that "X" is common and "Y" is less common.

4.'4+' indicates strong positivity, but due to quality issues with the serum, it may not actually reach '4+'; '1~3+' indicates that agglutination can be weak or strong.

Fig. S14. a) The detection results of two subtype (A_3B+) in 3D-μSTADs, indicating the high compatibility for weak blood sample in our system. b) The RBC antigen profile of A_3B+.

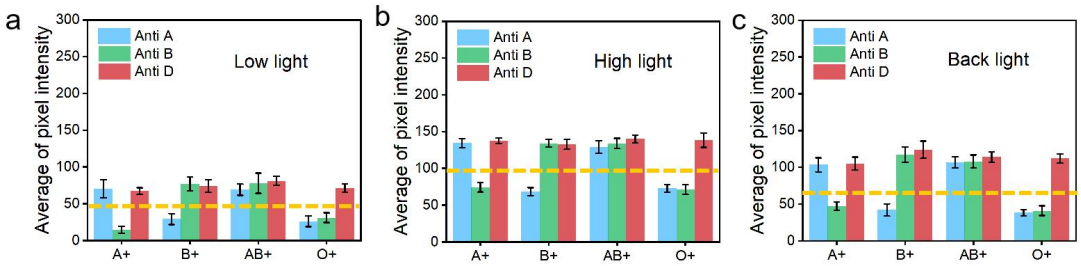


Fig. S15. The average of pixel intensity of the detection results across different environment light intensities (n=5), including low light a), high light b), and back light c). The results showed that each of these light intensity exhibited different thresholds (yellow dashed line) of the pixel intensity, indicating a significant impact of the environment on the detection results.

Method	Cost	Time	Portability	Centrifuge
Slide test	++	2-5 min	Yes	Not required
Tube test	++	10-30 min	No	Required
Gel card	+++	10-45 min	No	Required
Microplate	+++	10-30 min	No	Not required
3D μSTADs	+ (0.41 $)	2 min	Yes	Not required

Table S1. The comparison of our work with conventional blood typing methods. Our devices stands out at it extreme low cost, low detection time, and portability. These value were reported by Kai et al. [Front. Med, 2022, 9(22): 827619.] and Quraishy et al. [Adv. Clin. Chem. 2016, 77, 221-269].

Item	Cost (USD)
PDMS (~ 2 g)	$0.2
Cotton thread (~5 cm×3)	$0.01
Antibodies (~22.5 μL×3)	$0.2
Total	**$0.41**

Table S2. The cost of our proposed 3D-μSTADs. The extremely low cost possesses the potential to replace traditional blood typing method for remote rural areas in POCT cases.

Blood type	Number	Sensitivity(%)	Specificity(%)	Accuracy(%)
A+	26	100	100	100
B+	29	100	100	100
AB+	24	100	100	100
O+	22	100	100	100
A-	4	100	100	100

Table S3. Summary of blood typing results for 105 blood samples in 3D-μSTADs.

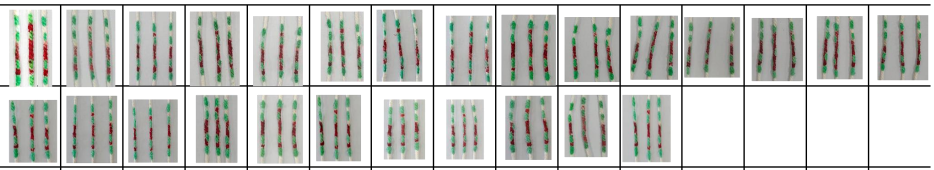
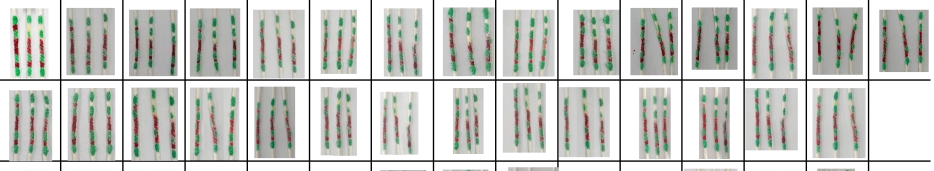
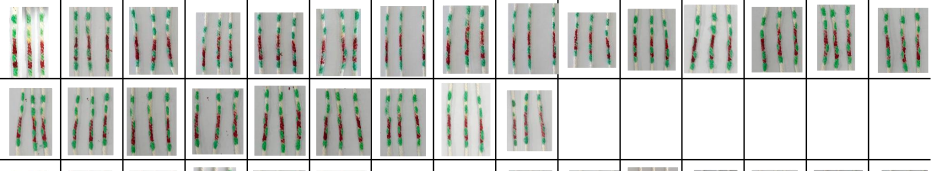
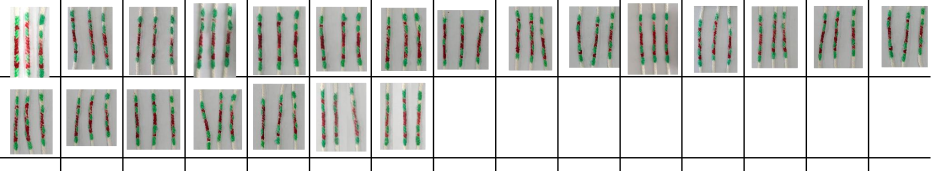
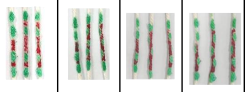
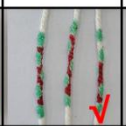
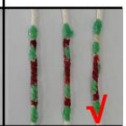
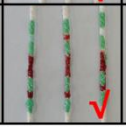
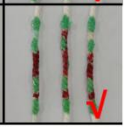
Blood type	Interpreted patterns	Raw detection results
A+		
B+		
AB+		
O+		
A-		

Table S4. 105 raw images of the blood typing results in 3D-μSTADs.

a

Blood Type	Interpreted pattern	Ambient condition: Temperature: 20.2 ℃ Humidity: 20%	Temperature: 20.2 ℃ Humidity: 60%	Temperature: 20.2 ℃ Humidity: 80%
O +				
A +				

b

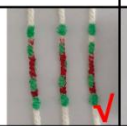
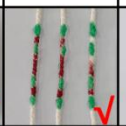
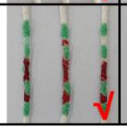
Blood Type	Interpreted pattern	Ambient condition: Temperature: 7.4 ℃ Humidity: 20%	Temperature: 20.2 ℃ Humidity: 20%	Temperature: 32.6 ℃ Humidity: 20%
O +				
A +				

Table S5. a) The blood typing results of blood sample in different temperature condition. b)The blood typing results of blood sample in different humidity condition.

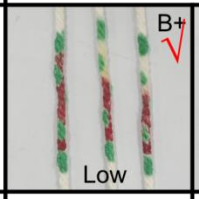
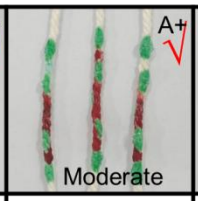
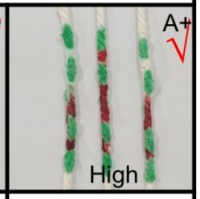
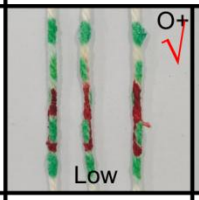
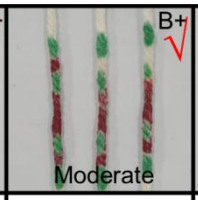
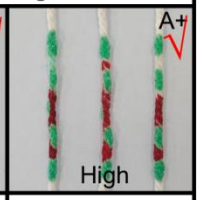
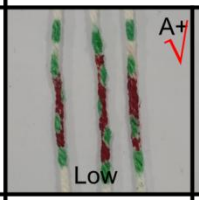
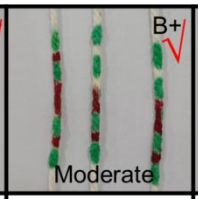
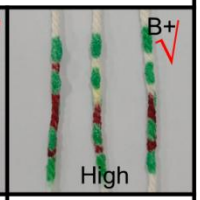
	Total cholesterol reference value: 2.34 - 5.7 mmol/L		
Blood typing results			
Value	**1.8 mmol/L**	**5.1 mmol/L**	**8.2 mmol/L**
	Total protein reference value: 60 - 80 g/L		
Blood typing results			
Value	**57.9 g/L**	**74.6 g/L**	**85.4 g/L**
	Blood glucose reference value: 3.9 - 6.1 mmol/L		
Blood typing results			
Value	**3.1 mmol/L**	**5.5 mmol/L**	**14.7 mmol/L**

Table S6. The blood typing results of blood sample with various total cholesterol, total protein and blood glucose concentrations. And all of these samples were accurately classified.