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

Supporting Information

An electricity- and instrument-free infectious disease sensor based on a 3D origami paper-based analytical device

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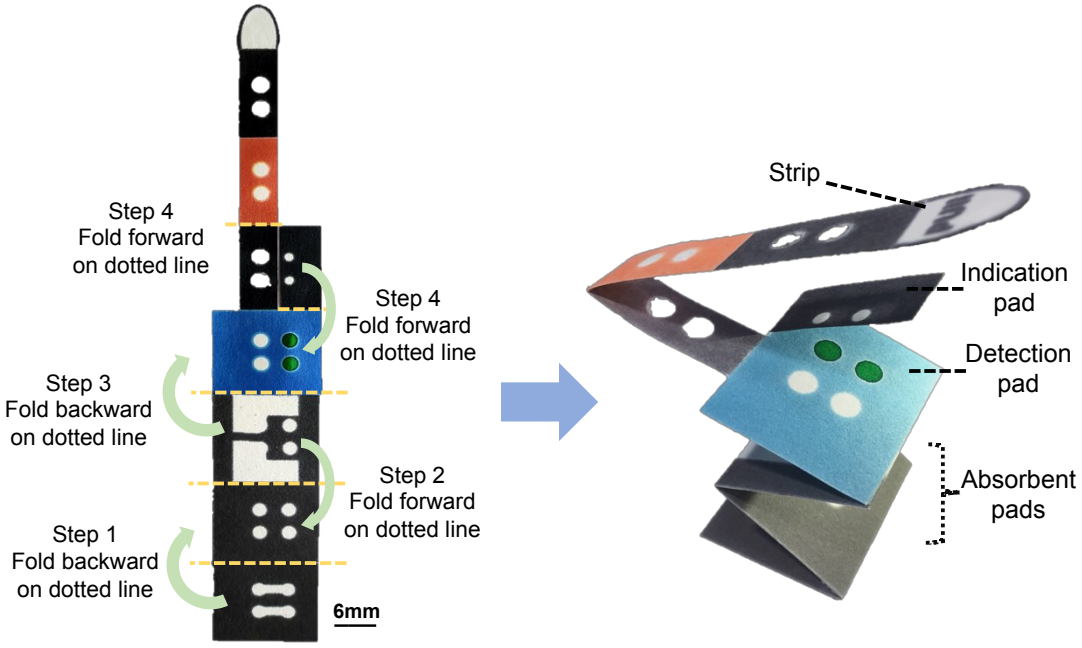

Fig. S1 Step-by-step origami folding instructions. The device is constructed from a single sheet of paper, in which hydrophilic wells and channels are connected to enable solution flow between the different layers of the final folded device. To perform the assay, the hydrophilic wells and channels on the pads and strip are modified with dried ELISA reagents that sequentially dissolve into the added sample solution or washing buffer as it moves through the folded device, with the final result shown on the detection pad.

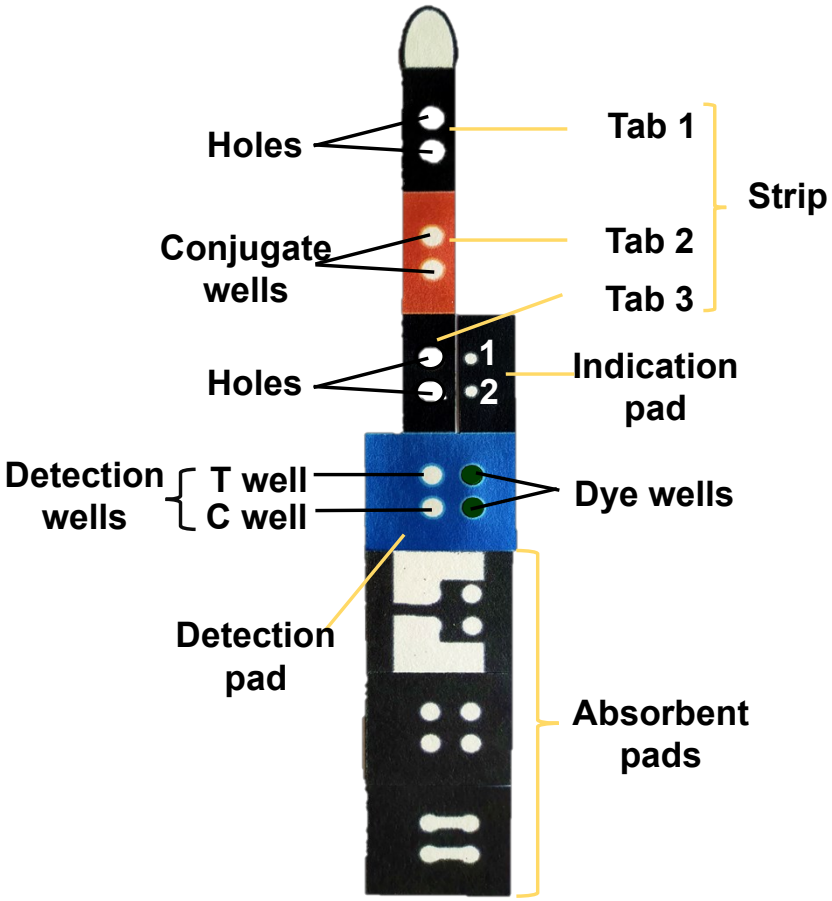

Fig. S2 The structure of the proposed 3D-tPADs. The T and C wells are modified with capture antibody and used for the sample and control solutions, respectively. The conjugate wells contain dried detection antibodies. The dye wells contain dried green food dye.

Table S1 The sucrose deposition parameters

Concentration (mg/mL)	Volume (μL)	Area (mm^2)	Mass (mg)
10	3	128.6	1.9
20	3	128.6	3.8
30	3	128.6	5.8
40	3	19.6	1.1
40	3	39.3	2.3
40	3	89.3	5.4
10	5	128.6	3.2
20	5	128.6	6.4
30	5	128.6	9.7
10	7.85	128.6	5.0
20	7.85	128.6	10.1
30	7.85	128.6	15.2
10	10	128.6	6.4
20	10	128.6	12.9
30	10	128.6	19.4

Table S2 The pullulan deposition parameters

Concentration (mg/mL)	Volume (μL)	Area (mm^2)	Mass (mg)
0.01	3	128.6	0.0019
0.05	3	128.6	0.0097
0.1	3	128.6	0.01948
0.5	3	128.6	0.09742
1	3	128.6	0.194848
5	3	128.6	0.974242
0.01	5	128.6	0.003247
0.05	5	128.6	0.016237
0.1	5	128.6	0.032475
0.5	5	128.6	0.162347
1	5	128.6	0.324747
3	5	128.6	0.974242
5	5	128.6	1.623737
0.01	7.85	128.6	0.005099
0.05	7.85	128.6	0.025493
0.1	7.85	128.6	0.050985
0.5	7.85	128.6	0.254927
1	7.85	128.6	0.509854
3	7.85	128.6	1.529561
5	7.85	128.6	2.549268
0.01	10	128.6	0.006495
0.1	10	128.6	0.064949
0.5	10	128.6	0.324747
1	10	128.6	0.64949
3	10	128.6	1.94848

5	10	128.6	3.24747

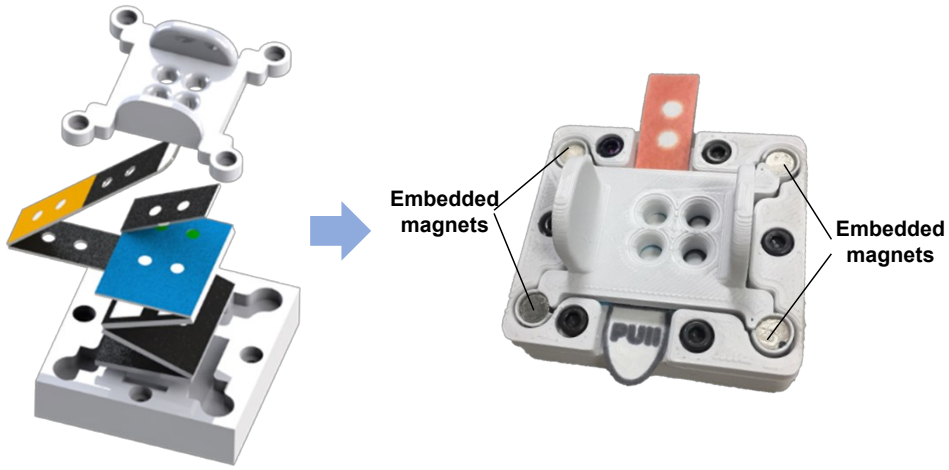

Fig. S3 Schematic and photograph of the 3D-printed sample holder containing the 3D-tPADs. The bottom substrate and top cover of the holder each feature 4 embedded magnetic disks to securely hold the 3D-tPADs in place by magnetic force.

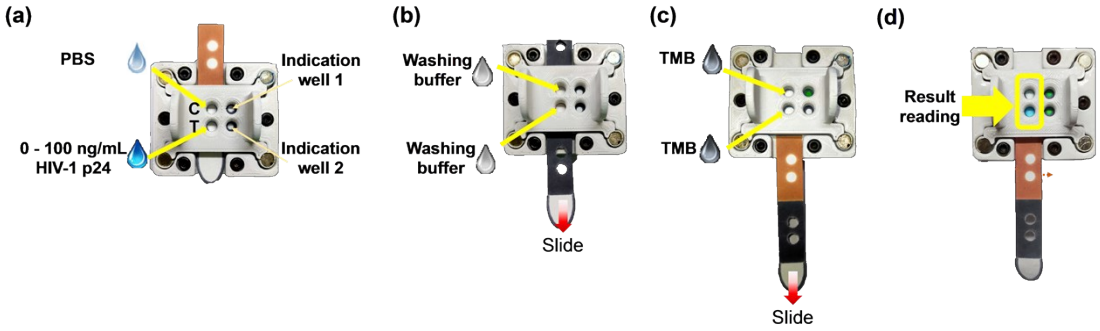

Fig. S4 The HIV-1 p24 detection process. (a) Sample solution containing HIV-1 p24 and PBS containing no HIV-1 p24 was added to the T and C wells, respectively. (b) We then slide the strip down to expose the conjugate wells and add washing buffer to each well. (c) When the indication well 1 turns green, we slide the strip down again to expose the C and T wells and add TMB solution to each well. (d) When the indication well 2 turns green, we observe the color of each well to obtain the test result reading.

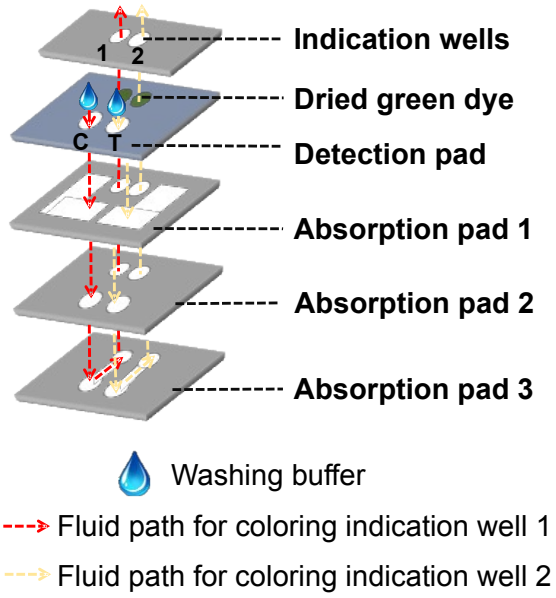

Fig. S5 The schematic of the fluid paths for coloring indication well 1 and 2.

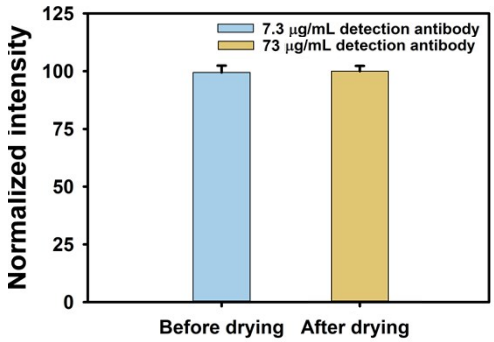

Fig. S6 The normalized color intensity of the paper-based device with different concentrations of liquid and dried detection antibody using TMB assay.

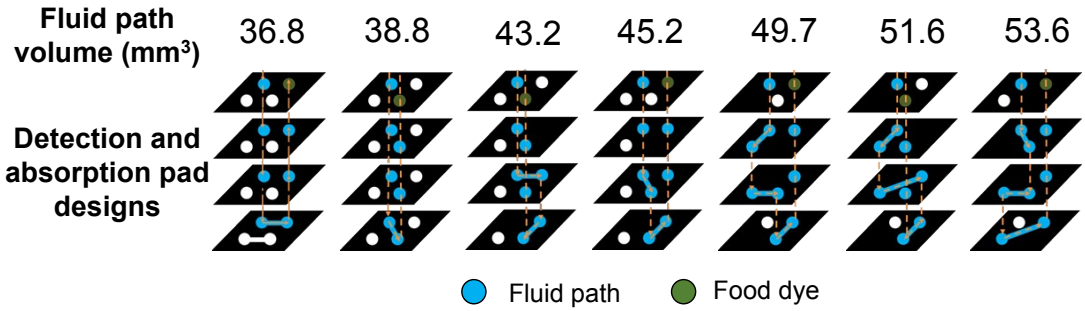

Fig. S7 Schematic of the absorbent and detection pad designs with different fluid path volumes.

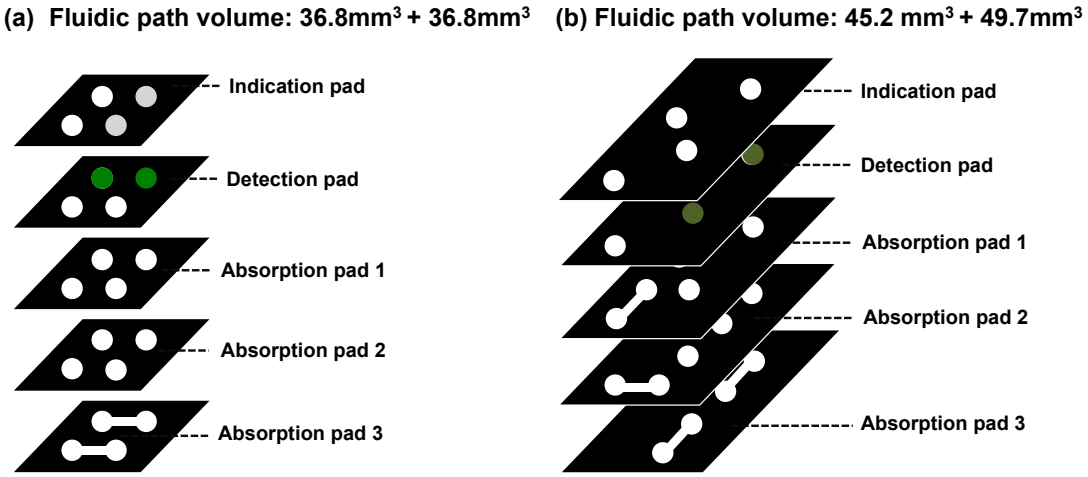

Fig. S8 Schematic of the indication, absorbent, and detection pads when the fluidic path volumes are (a) 36.8 mm^3 + 36.8 mm^3 and (b) 45.2 + 49.7 mm^3. The size of the pads with a fluidic path volume of 49.7 mm^3 is two-times larger than the size of the pads with a fluidic path volume of 36.8 mm^3.

Table S3 Comparison among the proposed assay and other related assays

	Detection limit (ng/mL)	Detection range (ng/mL)	Vendor/Reference
Laboratory ELISA	0.01	0.01 ~ 1	Sino Biological
Lateral flow assay & Fluorescent immunoassay	0.03	0.03 ~ 1	[1]
Paper-based device & Electrochemical assay	0.3	0.3 ~ 10^4	[2]
Plastic chips & ELISA	0.19	0.19 ~ 80	[3]

Proposed assay	0.01	0.01 ~ 30

References

1. Q. Wang, M. Hou, L. Liu, J. Ma, X. Zhang, Z. Zhou, Y. Cao, *Biomed. Environ. Sci.,* 2020, **33**, 174–182.

2. C. Zhao, X. Liu, *Biomicrofluidics,* 2016, **10**, 24119.

3. F. Li, H. Li, Z. Wang, J. Wu, W. Wang, L. Zhou, Q. Xiao, Q. Pu, *Sens. Actuators B Chem.,* 2018, **271**, 189–194.