Electronic Supporting Information

3D-printed devices for multiplexed semi-quantitative competitive lateral flow immunoassays

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1. Design of device type B

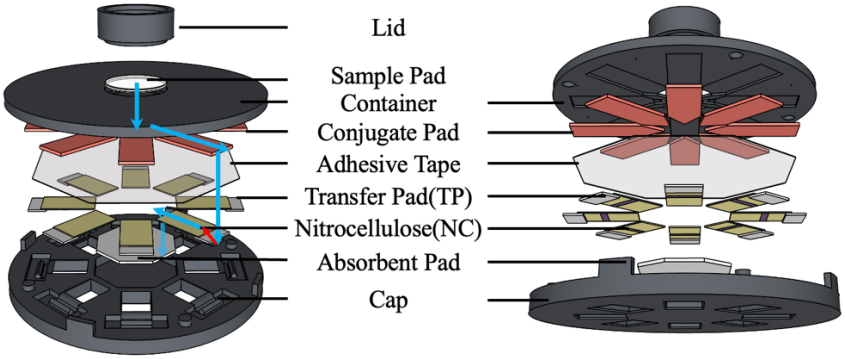


Fig. S1 Design of device type B with 8 LFIA lanes for plastic-backed nitrocellulose membranes; blue/red arrows represent the sample liquid flow path on the example of a single LFIA lane.

2. Details and layouts of all pads

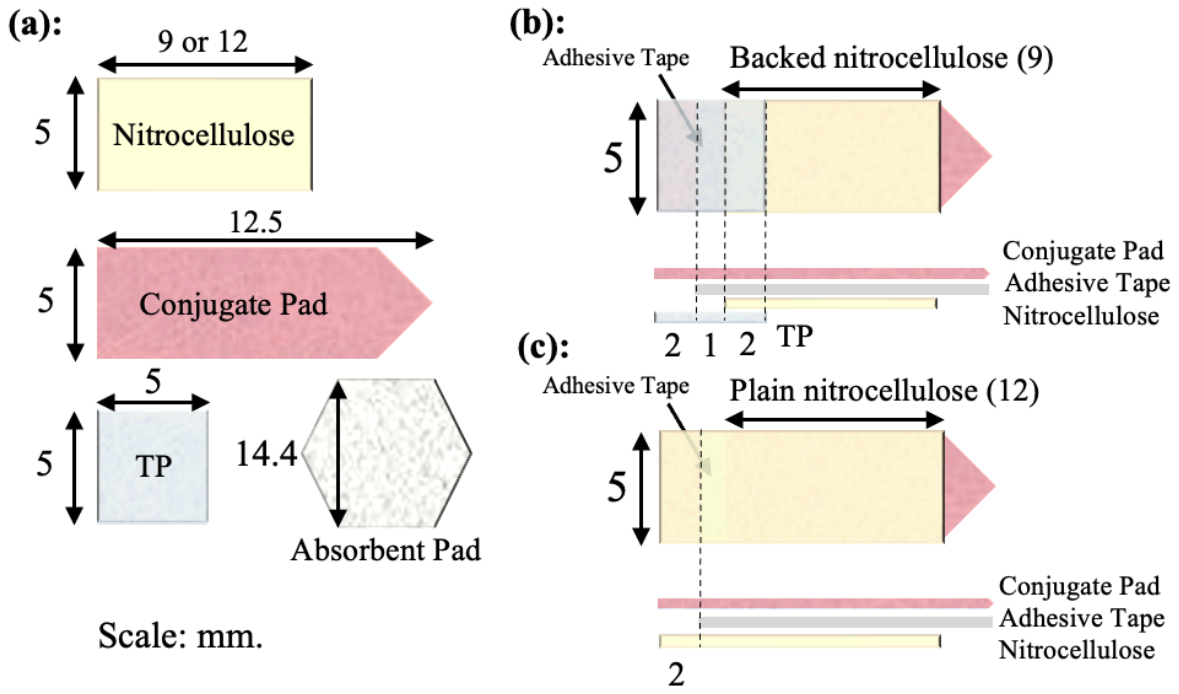
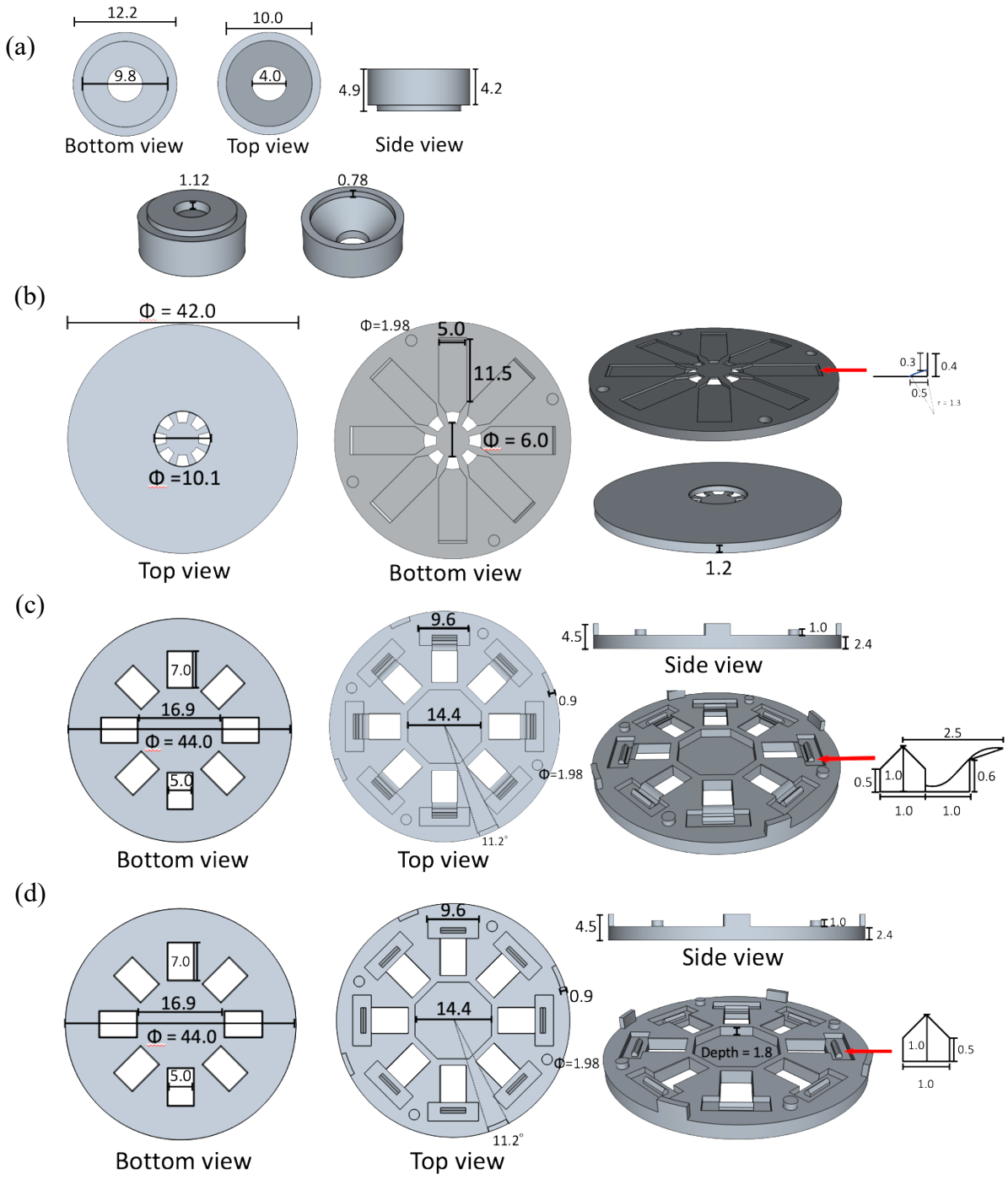
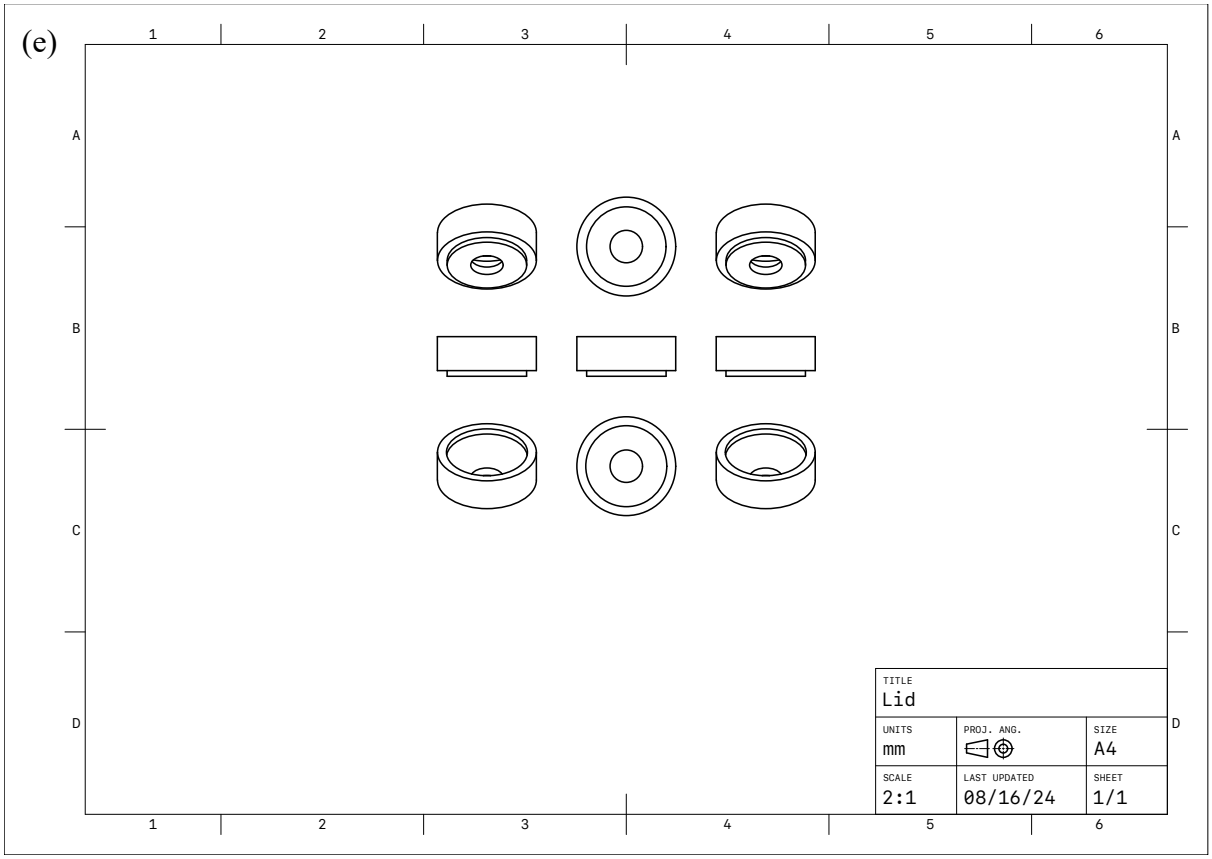


Fig. S2 Details of size and arrangement of all components used for LFIA preparation in each lane: (a) cutting dimensions of all pads (TP: transfer pad); pad arrangement for device types B (b) and P (c); all numerals indicate dimensions in mm.

3. Details and design sketches of 3D models



(e)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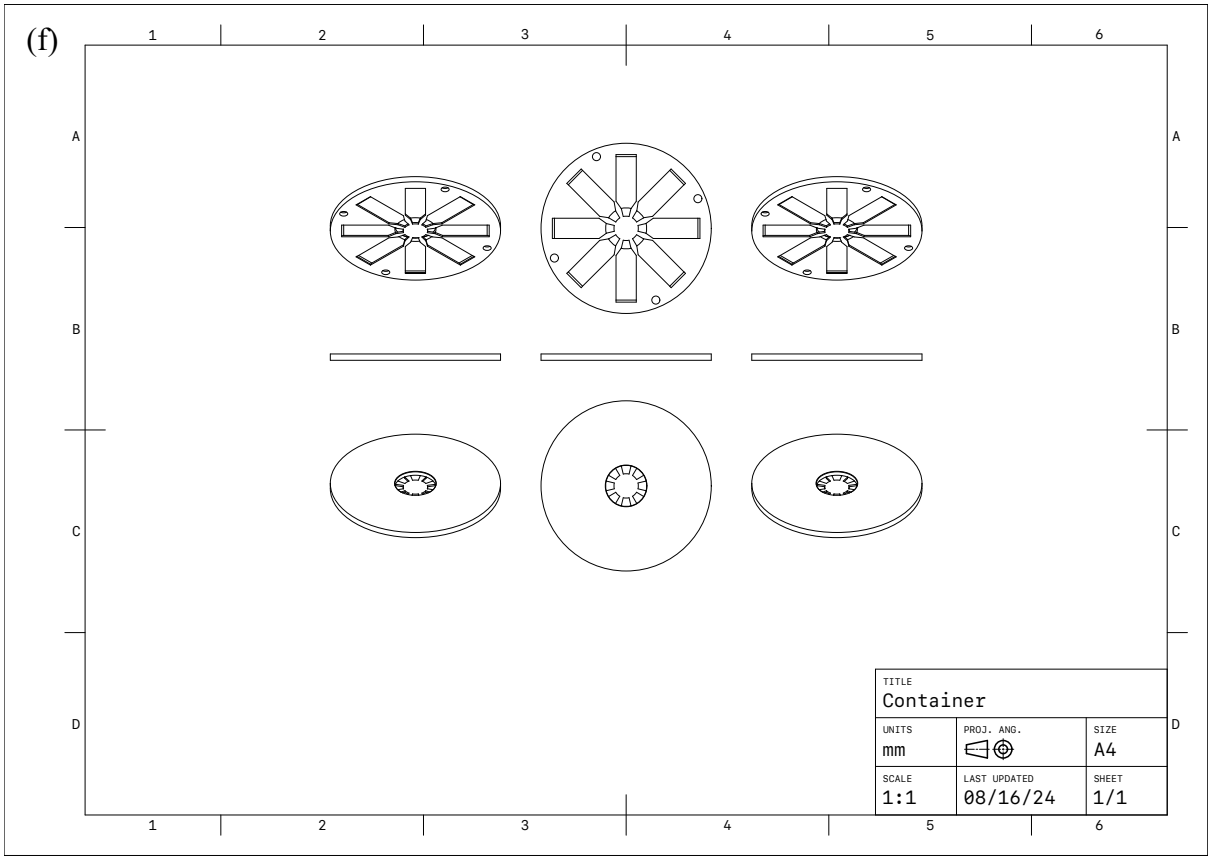
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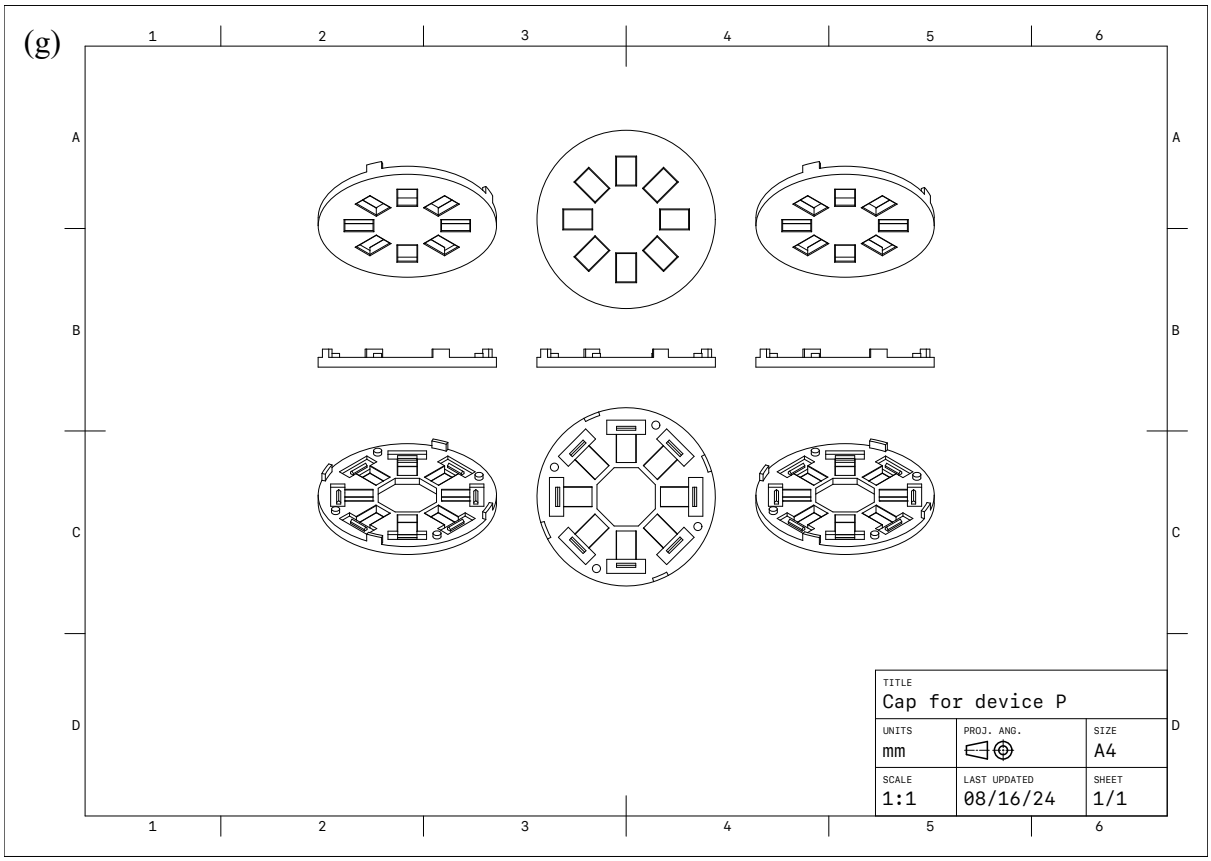


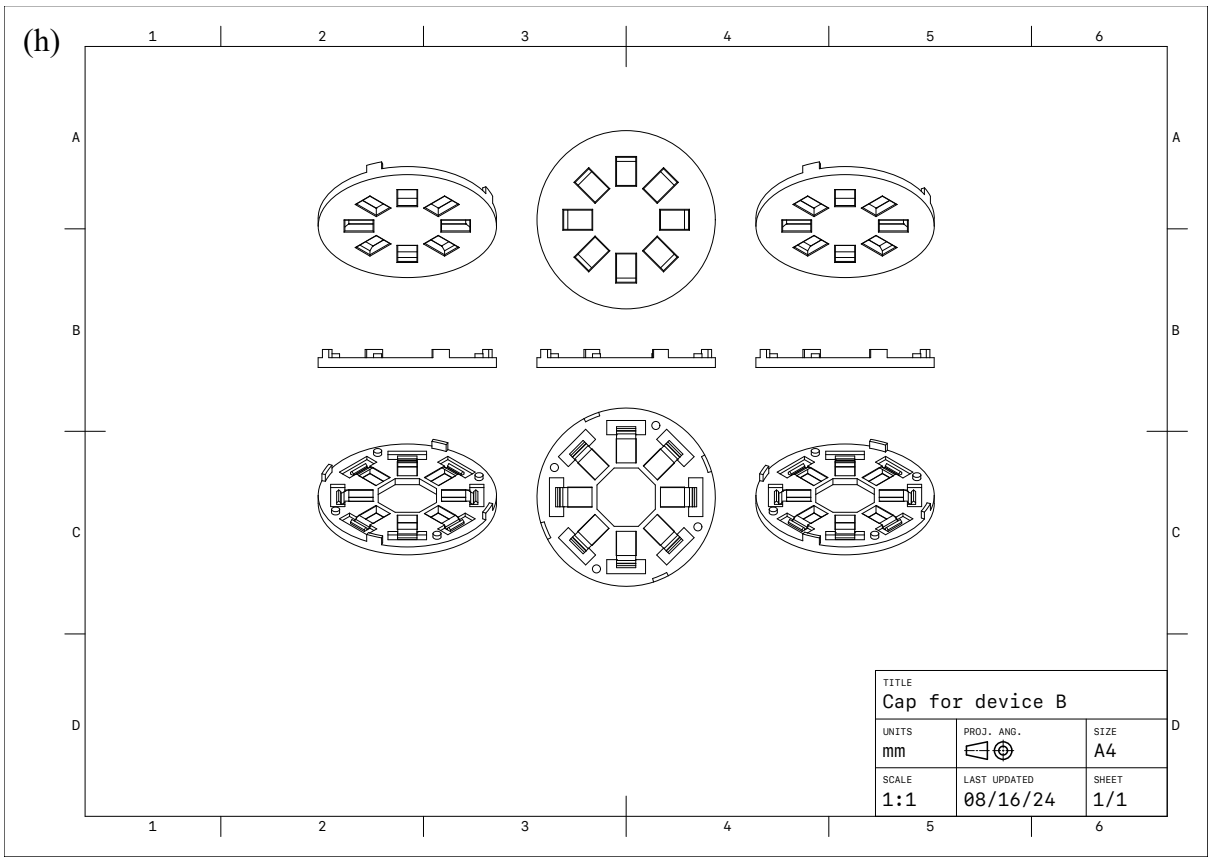


Fig. S3 Sketches for Lid (a, e), Container (b, f), Cap for device P (c, g), and Cap for device B (d, h); all numerals indicate dimensions in mm.

4. Design of cap for device type B and P

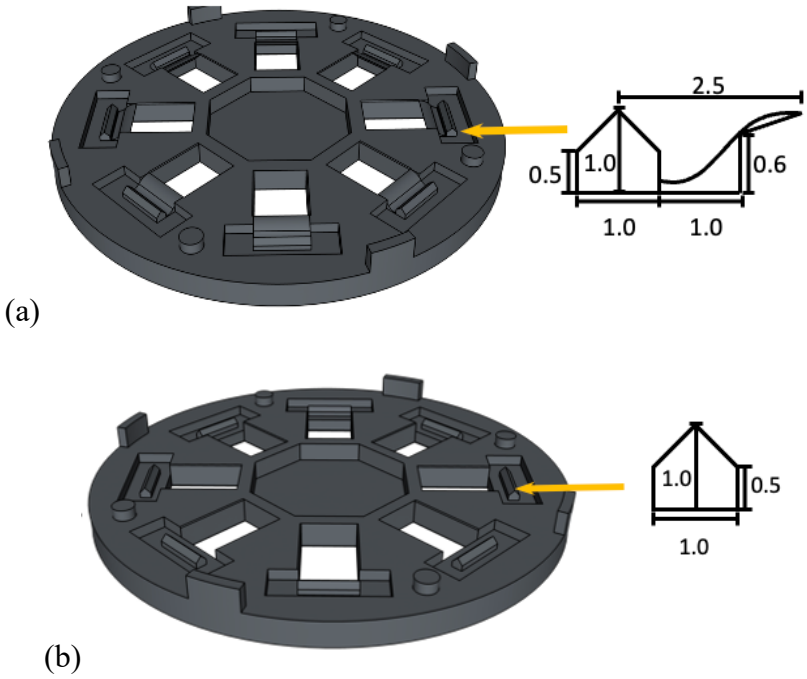
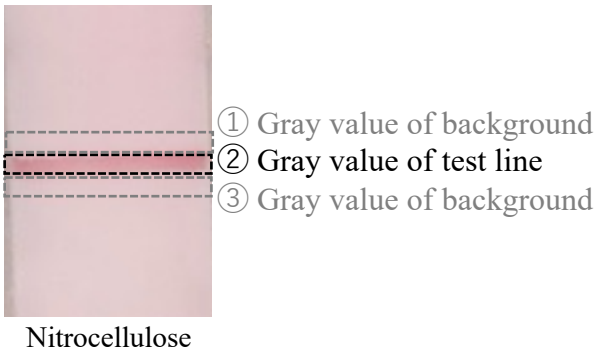


Fig. S4 Details of cap supporting protrusion sections for device type B (a) and type P (b); all numerals indicate dimensions in mm.

5. Definition of ΔGray value



ΔGray value = (①+③)/2 - ②

Fig. S5 Definition and calculation method of the ΔGray value used for quantitative analysis of test line color intensities.

6. Original scanned picture for text-displaying semiquantitative detection

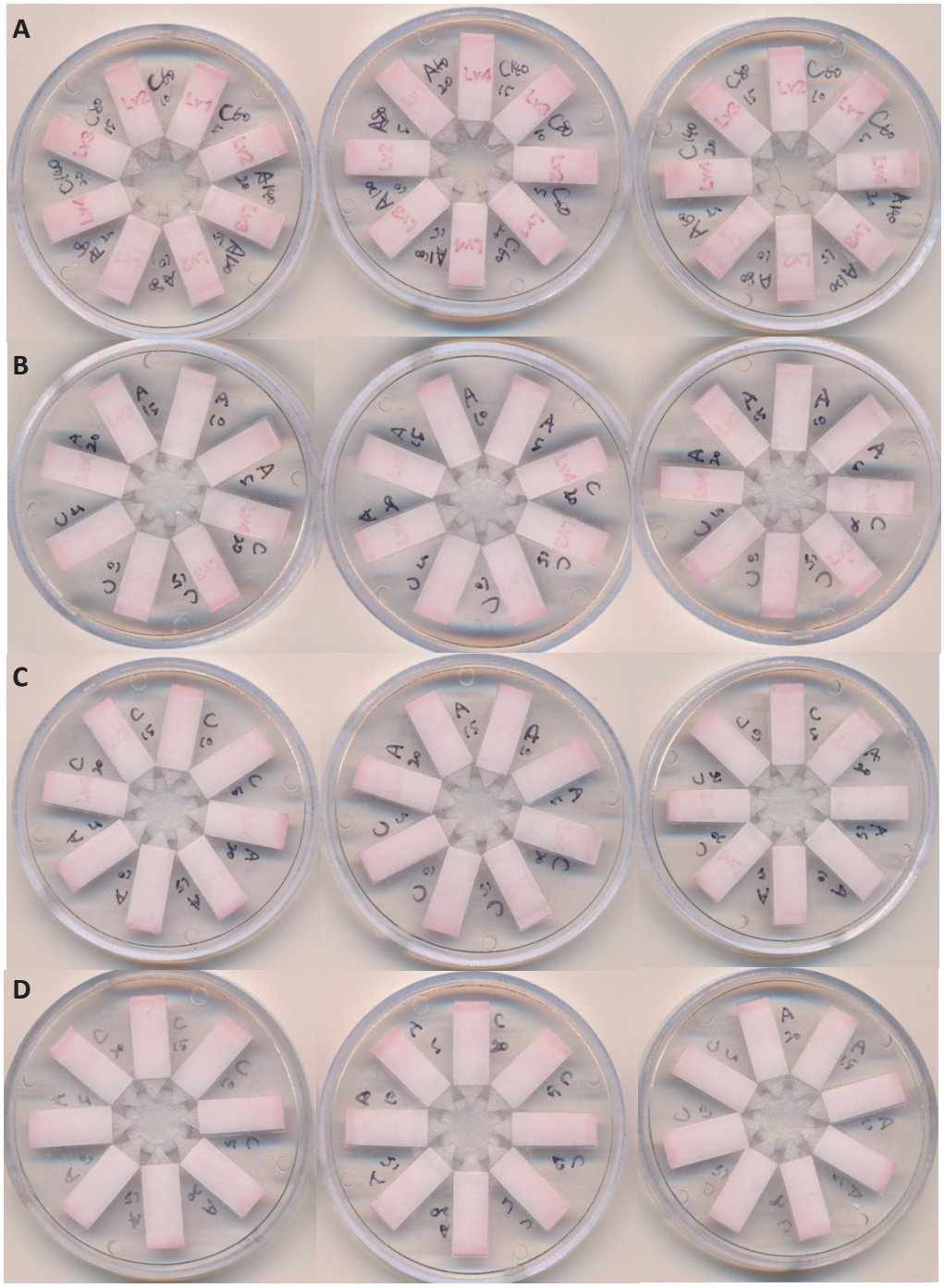


Fig. S6 Unmodified color scans showing text-displaying results for the semi-quantitative detection of (A) caffeine = 0 ng/mL and acetaminophen = 0 ng/mL, (B) caffeine = 4 ng/mL and acetaminophen = 4 ng/mL, (C) caffeine = 10 ng/mL and acetaminophen = 8 ng/mL, (D) caffeine = 175 ng/mL and acetaminophen = 12 ng/mL in artificial urine samples; all experiments have been performed in triplicate with the results of 3 devices shown in each row.

7. Composition of artificial urine

Table S1 Composition of artificial urine samples

Chemicals	Conc.(mM)
Lactic Acid	1.1
Citric Acid	2
Sodium Bicarbonate	25
Urea	170
Calcium Chloride	2.5
Sodium Chloride	90
Magnesium Sulfate	2
Sodium Sulfate	10
Potassium Dihydrogen Phosphate	7
Dipotassium Hydrogen Phosphate	7
Ammonium Chloride	25
Uric Acid	0.4
Creatinine	7
BSA	1(g/L)

8. Relationship between combined ratio and initial concentration of Ab and Ag

$$Ab + Ag \underset{\rightleftarrows}{K_a} Ab\text{-}Ag$$

$$K_a = \frac{C}{[\mathrm{Ab}][\mathrm{Ag}]} = \frac{C}{([\mathrm{Ab}]_0 - C)([\mathrm{Ag}]_0 - C)}$$

$$C^2 - \left(\frac{1}{K_\mathrm{a}} + [\mathrm{Ab}]_0 + [\mathrm{Ag}]_0\right) C + [\mathrm{Ab}]_0[\mathrm{Ag}]_0 = 0$$

$$C = \frac{\left(\frac{1}{K_\mathrm{a}} + [\mathrm{Ab}]_0 + [\mathrm{Ag}]_0\right) - \sqrt{\left(\frac{1}{K_\mathrm{a}} + [\mathrm{Ab}]_0 + [\mathrm{Ag}]_0\right)^2 - 4[\mathrm{Ab}]_0[\mathrm{Ag}]_0}}{2}$$

- K_a : Affinity constant($\approx 10^{12}$)
- [Ab] : Antibody conc.
- [Ag] : Antigen conc.
- [Ab-Ag] : Complex conc.(C)
- $[\mathrm{Ag}]_0$: Initial antigen conc.
- $[\mathrm{Ab}]_0$: Initial antibody conc.
- **Combined ratio[%]** $= C / [\mathrm{Ab}]_0 \times 100\%$

Eq. S1 Relationship between immunocomplex and the concentration of antibody or antigen