Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary information

Cotton Fabric as Immobilization Matrix for Low-Cost and Quick Colorimetric Enzyme-Linked Immunosorbent Assay (ELISA)

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Conventional ELISA Protocol and Calibration

Free-ß-human chorionic gonadotropin ELISA kit consisted of a sandwich ELISA method, where the primary antibody was already immobilized on the 96 microtiter well plate. 50 µL of hCG standards (2.5, 5, 10, 25 and 50 ng/mL) and 100 µL of PBS were added to each well and incubated at 37°C for 30 min. Subsequently, 150 µL of enzyme conjugated reagent was added and incubated under the same conditions. Following each addition, the plate was washed 5 times with deionized water and vigorously flicked to remove the unbound reagents. 100 µL of TMB reagent were then added into each well and incubated for 20 min and the colorimetric reaction stopped with the addition of 100 µL of 1 N of HCl solution. The absorbance values were determined within 15 min from the change of color from blue to yellow using a microtiter plate reader at 450 nm wavelength (Alpco Immunoassay Diagnosis, USA).

The average of absorbance for each hCG standard was plotted versus the number of mols of antigen (calculated from the concentration using molecular mass data of chorionic gonadotropin subunit beta of 17,139 Da ((UniProtKB) 2013)) and the data was fitted with both a sigmoid function (R^2 = 0.998, n =3) and a linear function (R^2 = 0.995, inset) (Fig. S1). This implies that the conventional ELISA for hCG test provides a linear range up to concentrations of 146x10^{-6} nmols or 50 ng/mL.

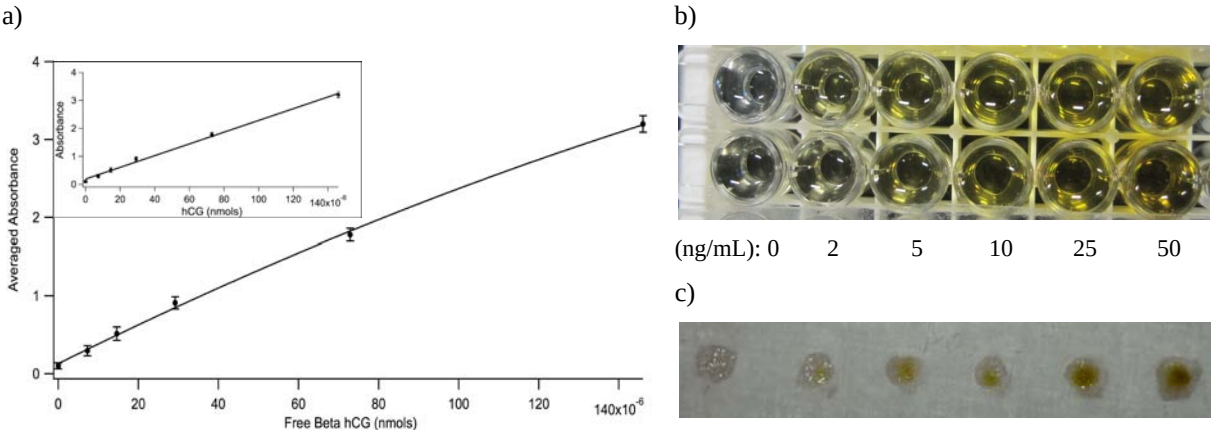

Fig. S1 ELISA calibration. a) Calibration curve for conventional ELISA, the absorbance values were plotted versus the number of nmols of antigen and the data fitted with a sigmoid function R^2 = 0.999, while the linear function in the inset presents a R^2 = 0.996 (data points are represented by mean ± stdev, n = 3); b) Image of the microtiter plate wells during a conventional ELISA calibration; c) Image of the microzones during cloth-based ELISA calibration. The different concentrations of antigen (0 to 50 ng/mL) are discerned by different color intensity.

CADs Fabrication and Analysis

The fabrication of the CADs consists of a scouring pretreatment of the cloth by washing it in a soda ash bath for 5 min at 100°C. The scouring treatment allows the removal of the natural wax of the cloth and hence increases the wicking properties of the material. In addition, this increases the absorbability of the reagent on the cloth. The design required for the diagnosis test zones was drawn using Graphtec Silhouette software and printed onto paper with a cutter printer. The paper was then dipped into melted wax, which is typically prepared using a mixture of bees-paraffin waxes to provide different properties to the wax, and allowed to dry for a few minutes at room temperature (25°C). As a result, the wax patterning paper was created with hydrophilic and hydrophobic delimited areas. The wax was then transferred to the cloth by manually pressing both platforms together and applying heat to the wax pattering paper with a soldering device (Fig. S2).

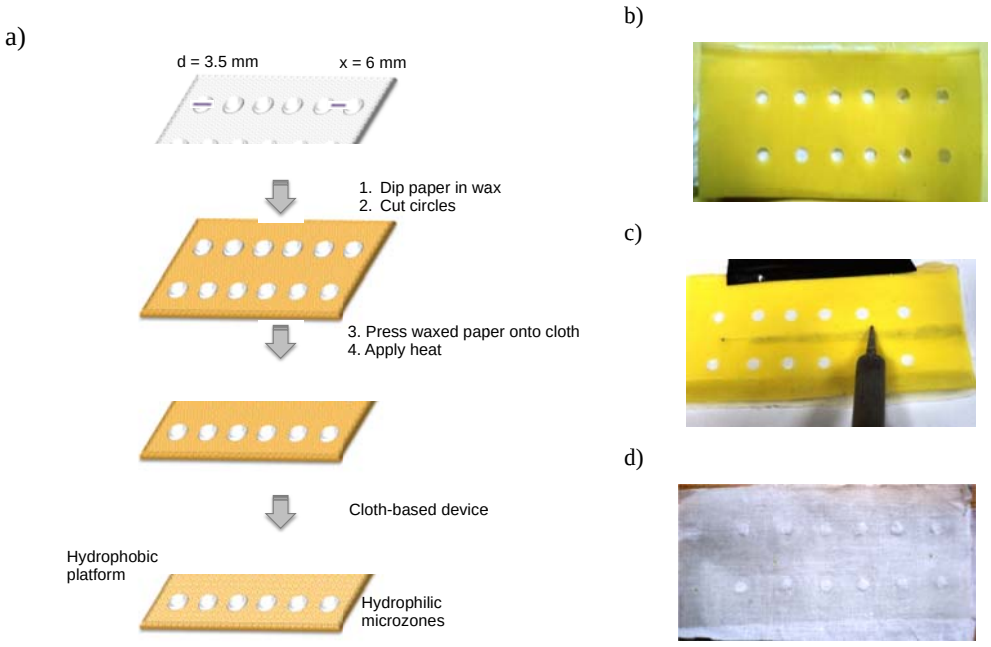

Fig. S2 Fabrication of cloth-based ELISA microzones. a) Schematic representation of the fabrication of the wells. The paper was designed with two rows and six columns of circles (d = 3.5 mm) separated by a distance of 6 mm between each other. This was then dipped into melted wax and allowed to dry for a few minutes at room temperature. The circles designed on the paper were cut and the wax was then transferred to the cloth by heat application. The wax on the cloth provides a hydrophobic platform delimiting the circles as hydrophilic microzones for the ELISA assay. b) Waxed-paper. c) Transferring of the wax onto the cloth by heat application. d) Waxed-cloth.

CADs Analysis

The intensity of the colorimetric reaction was analyzed using snapshots of the assay. However, the water reflection in the microzones affected these measurements. If let to dry, a relatively extended period of time, during which the TMB color saturated and HCl color disappeared, was required. To overcome this inaccuracy due to the water reflection the images were converted to grey scale and inverted to analyze the intensity of the lighter area. The relative mean intensity of each standard was obtained from the ratio of the standards absolute mean intensity and the buffer absolute mean intensity measurements. The relative intensity was plotted versus the concentration of standard and calibration curves were fitted using a modified Hill equation, a sigmoidal function with different shape than Hill's equation (Equation 1).

$$I = I_{min} + \frac{I_{max}}{1+\exp\left\{-\left(\frac{[S]-K_M}{\propto}\right)\right\}} \quad (1)$$

where, I_{min} is the minimum intensity, I_{max} is the maximum intensity, [S] is the concentration of substrate, α is the Hill coefficient and K_M is the Michaelis-Menten constant, the [S] value at $I_{max}/2$.

The Hill's equations for graphs in **Fig. 3** of the main text are:

Fig. 3a) TMB solution intensity:

- GA-immobilized CAD (blue solid line): (-11.368 + 15.396 / (1 + exp((x_0+7.6753e-05)/ 5.3969e-05))) with $R^2 = 0.997$
- Cellulose entrapment (no immobilization reagent, red dashed line): (-8.8403 + (12.536 / (1 + exp((x_0+1.0383e-04)/ 7.8369e-05))) with $R^2 = 0.983$

Fig. 3b) HCl solution intensity:

- GA-immobilized CAD (blue solid line): (-8.2304 + 11.521 / (1 + exp((x_0+6.3771e-05)/ 4.5307e-05))) with $R^2 = 0.968$
- Cellulose entrapment (no immobilization reagent, red dashed line): (-8.612 + 11.691 / (1 + exp((x_0+5.3518e-05)/ 3.4513e-05))) with $R^2 = 0.983$

Fig. 3c) Cellulose entrapped device:

- HCl (blue solid line): (-8.8403 + 0.229 / (1 + exp((x$_0$+1.0383 e-04)/ 7.8369e-05))) with R^2 = 0.99
- TMB (red dashed line): (-8.612 + 11.691 / (1 + exp((x$_0$+5.3528 e-05)/ 3.4513e-05))) with R^2 = 0.98

Cloth-based ELISA vs Conventional ELISA

The volume of the reagents and incubation time play an important role in increasing the efficiency and reducing the cost of the device. Hence, a clear advantage of cloth-based ELISA over the conventional method is the reduction of time and reagent volume required for the assay (Table S1).

Table S1 Comparison of cloth-based ELISA and conventional ELISA.

Time and reagents	**Cloth-based ELISA**		**Conventional ELISA**	
	Volume [µL]	Time [min]	Volume [µL]	Time [min]
1) Antibody Immobilization	3 µL	15 min	50-100 µL	Overnight (Thermo-Fisher-Scientific 2010)
2) Blocking	20 µL	20 min	300 µL	Overnight
3) Antigen	3 µL	15 min	100 µL	30 min (Alpco-Diagnostics 2011)
4) Enzyme-conjugate antibody	3 µL	15 min	150 µL	30 min
5) TMB reagent	3 µL	8 min	100 µL	20 min
6) Stop solution	Not needed	-	100 µL	30 min
Total Fabrication (Step 1-2)	23 µL	35 min	400 µL	1440min
Total assay per zone (Step 3-6)	9 µL	38 min	450 µL	110 min
Total Time	32 µL	73 min	850 µL	~ 1550 min

Reference:

Alpco-Diagnostics, 2011. Human Chorionic Gonadotropin ELISA. Alpco Immunoassays, p. For the quantitative determination of hCG in human samples. Alpco-Diagnostics, USA.

Thermo-Fisher-Scientific, 2010. ELISA technical guide and protocols. Technical Tip#65. Thermo Fisher Scientific Inc, USA.

(UniProtKB), P.K., 2013. P01233 (CGHB_HUMAN). Protein Knowledgebase (UniProtKB). UniProtKB/Swiss-Prot.