Electronic Supplementary Information for:

Highly sensitive colorimetric detection of glucose and uric acid in biological fluids using chitosan-modified paper microfluidic devices

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▪ Fabrication of µPADs

µPADs were produced by a stamping process. Briefly, a filter paper sheet previously impregnated with paraffin was placed over another filter paper without paraffin (native paper), creating a "sandwich" of papers. Then, a metal stamp preheated at 150°C was pressed against the paper sandwich in order to delimit hydrophobic barriers on native paper. The dimensions of µPADs were 45 mm × 45 mm, where a central zone (inlet sample) was interconnected by microfluidic channels (3 mm width and 10 mm long) with eight circular zones (detection zone) with 5 mm diameter each. A configuration of µPAD used in this study as well the incorporation process of chitosan followed by reaction scheme that occurs on detection zone are showed in Figure S1.

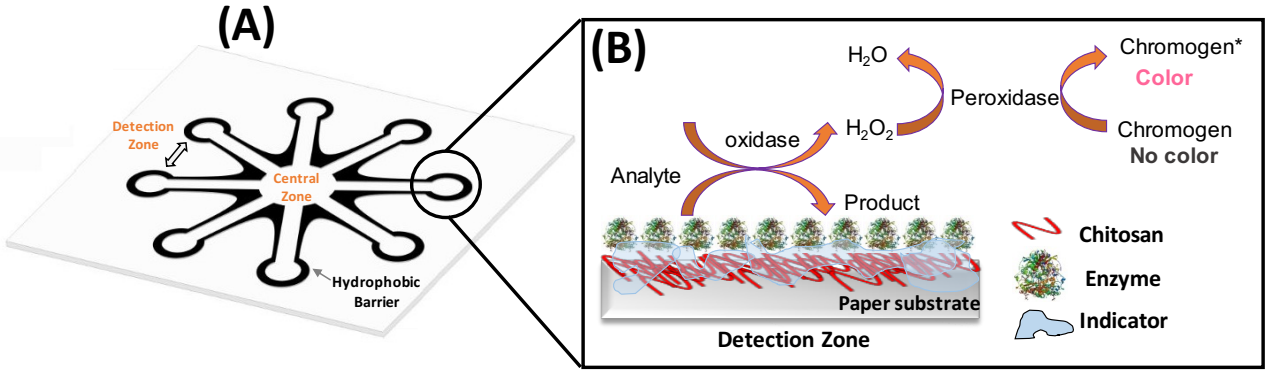

Figure S1 – Presentation of **(A)** layout of stamped µPADs and **(B)** scheme of reaction that occurs on detection zones after sample addition promoting color changes due to the oxidation of chromogen agent. In **(A)**, the layout of µPADs contains one central zone for sample inlet and eight detection zones interconnected by microfluidic channels.

- **Characterization Process**

- **FTIR**

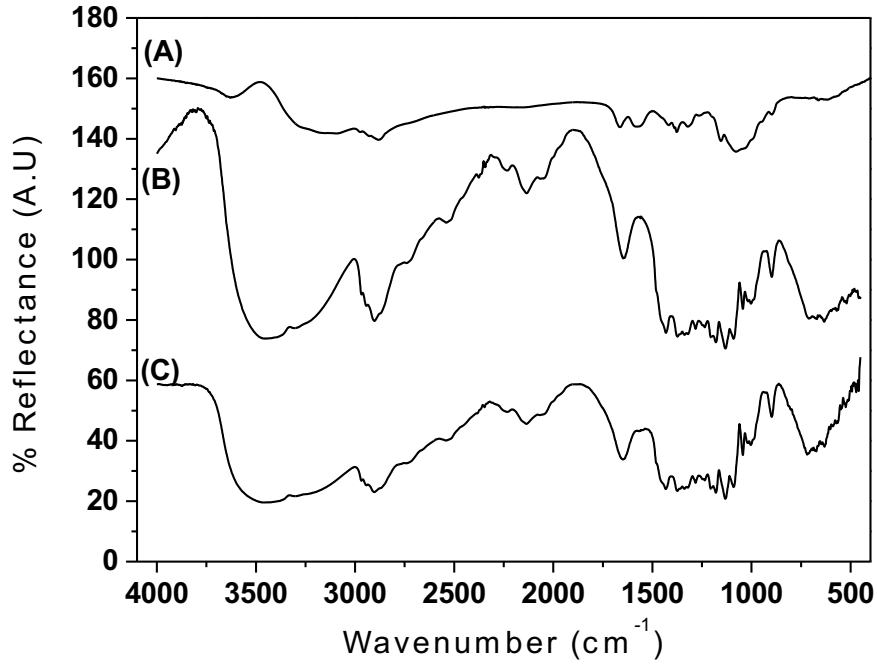

Figure S2 – FTIR spectra of **(A)** chitosan, **(B)** paper and **(C)** Paper + 0.5% (w/v) chitosan.

- **Effect of washing step with PBS and saline solutions**

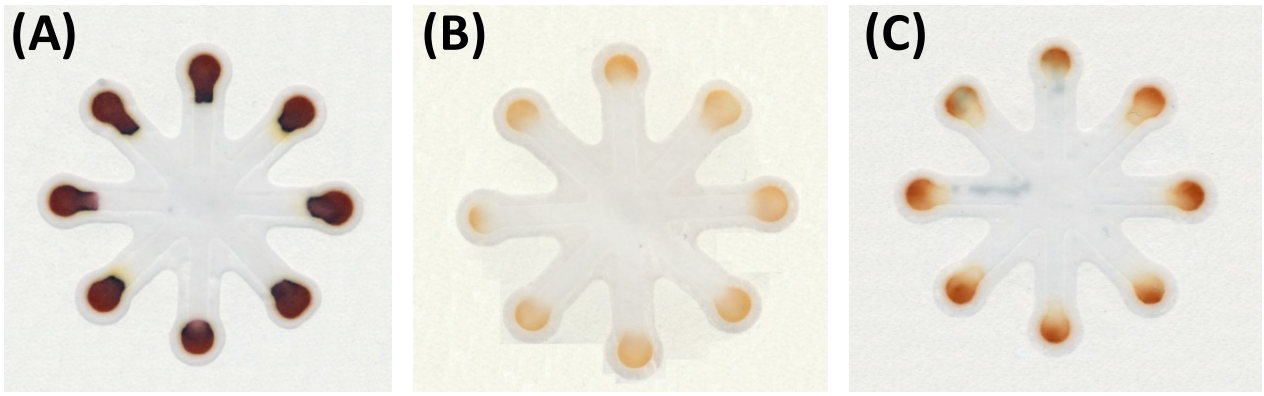

Figure S3– Effect of washing process with **(B)** PBS and **(C)** saline solutions. The washing step was realized after enzyme addition and color intensity was compared with non-washed µPAD **(A)**. The colorimetric readout was obtained for glucose bioassay at concentration of 3.0 mM using KI as colorimetric indicator.

- **Color Intensity and Gradient**

The color intensity and gradient obtained to optimization process of the chitosan incorporation on µPAD. The color gradient values represent the standard deviation of the color formed inside the detection zone. The higher color gradient, the greater heterogeneity of colorimetric response.

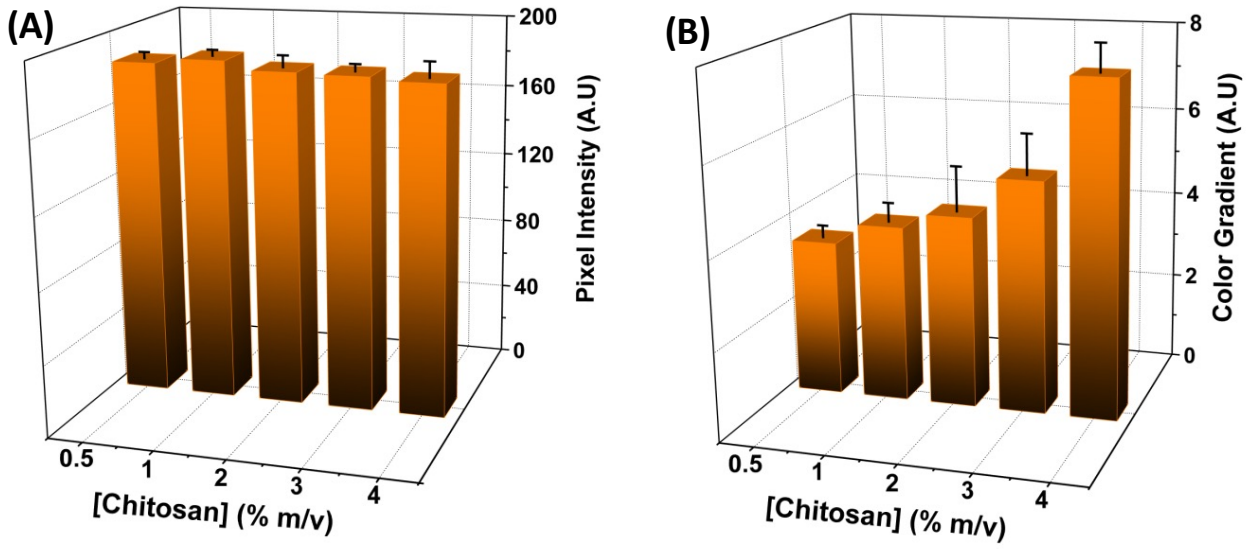

Figure S4- Effect of chitosan concentration on **(A)** color intensity and **(B)** color gradient developed inside detection zones. Other conditions: See Fig. S3.

- **Optimization of sample volume and reaction time**

The sample volume and time reaction were two different parameters studied on chitosan-modified μPADs. First, the determination of ideal sample volume was optimized ranging the volume added in the central zone from 45 to 80 μL. The effect of sample volume on color intensity response is evidenced in Figure S6.

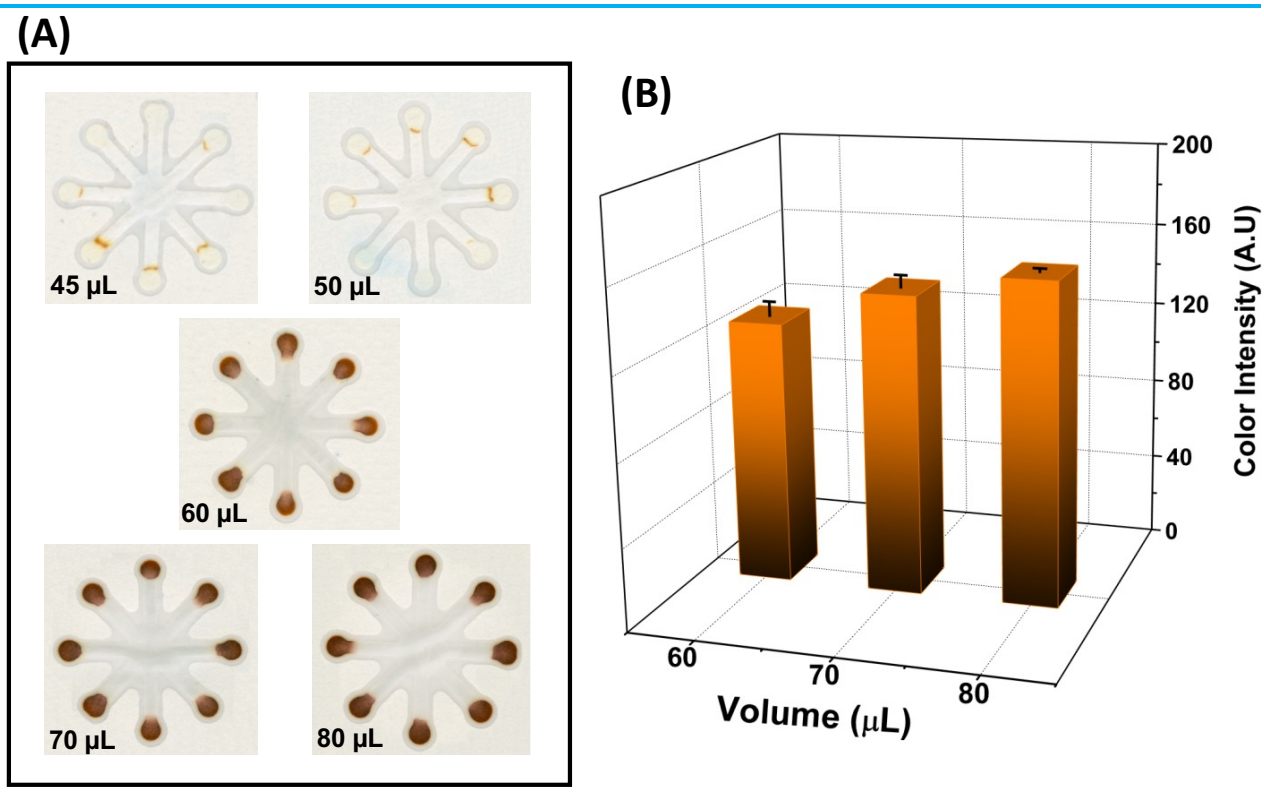

Figure S5- Effect of the sample volume on color intensity development inside detection zones previously modified with chitosan. **(A)** and **(B)** represent the color intensity and the optical images of μPADs showing the glucose assay ranging the volume from 45 to 80 μL, respectively. Other conditions: See Fig. S3.

The reaction kinetics was studied using glucose as model analyte. Colorimetric detection was performed scanning the image over time after glucose solution reach the detection zone. The reaction time was monitored from 5 to 30 min. Color intensity as function of time is demonstrated in Figure S8.

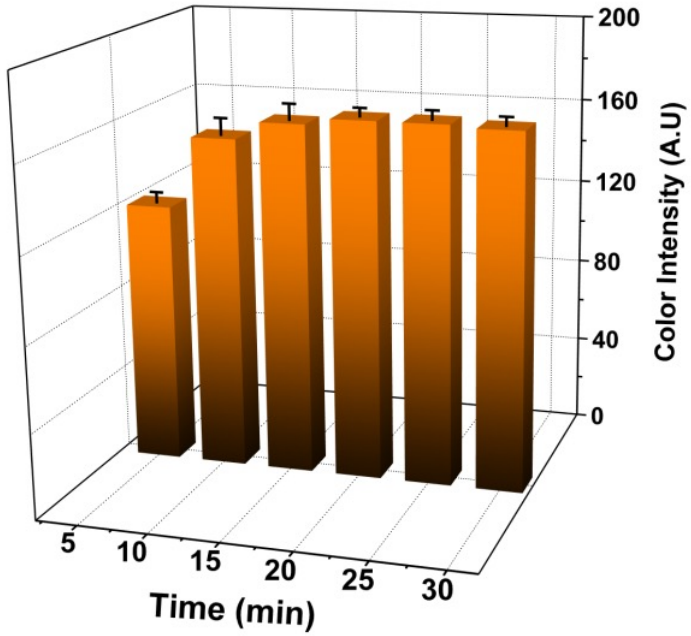

Figure S6. Effect of the reaction time on color intensity realized on chitosan-modified µPADs. Other conditions: See Fig. S3.

▪ Feasibility of chitosan-modified µPADs

Table S1- Values of limit of detection, sensitivity and linear range found for glucose and uric acid using different colorimetric indicators.

Analytes	Indicators	LD (µM)	Sensitivity (A.U./mM)	Linear range (mM)	R^2
Glucose	4-AAP/DHBS	23	86	0.1 - 1.0	0.984
	TMB	57	48	1.0 - 5.0	0.996
	KI	96	30	1.0 - 6.0	0.995
Uric Acid	4-AAP/DHBS	37	106	0.1 - 1.0	0.988
	OD	150	32	1.0 - 5.0	0.997

- **Equation of calibration curves**

Glucose:

$y_{4\text{-AAP/DHBS}} = 2.029 + 85.913 \times [\text{Glucose}]$

$y_{KI} = 2.006 + 29.577 \times [\text{Glucose}]$

$y_{TMB} = 0.226 + 48.566 \times [\text{Glucose}]$

UA:

$y_{4\text{-AAP/DHBS}} = 3.384 + 105.823 \times [\text{UA}]$

$y_{OD} = 0.0009 + 32.4644 \times [\text{UA}]$

Table S2. Comparison of the glucose and uric acid concentrations determined in two artificial human serum samples.

Serum Samples	Analytes	Reference Method (mM)	Our Device (mM)
Level I	Glucose	5.3 ± 0.6	4.9 ± 0.3
	Uric Acid	0.22 ± 0.05	0.20 ± 0.02
Level II	Glucose	13.7 ± 1.0	12.7 ± 0.2
	Uric Acid	0.55 ± 0.10	0.53 ± 0.02

*While artificial serum sample level I presents glucose and UA concentration ranges in normal conditions (4.5 – 5.8 mM for glucose and 0.12 – 0.38 mM for UA), artificial serum sample level II is related to biological disorders (12.4 – 16.2 mM for glucose and 0.43 – 0.58 mM for UA).

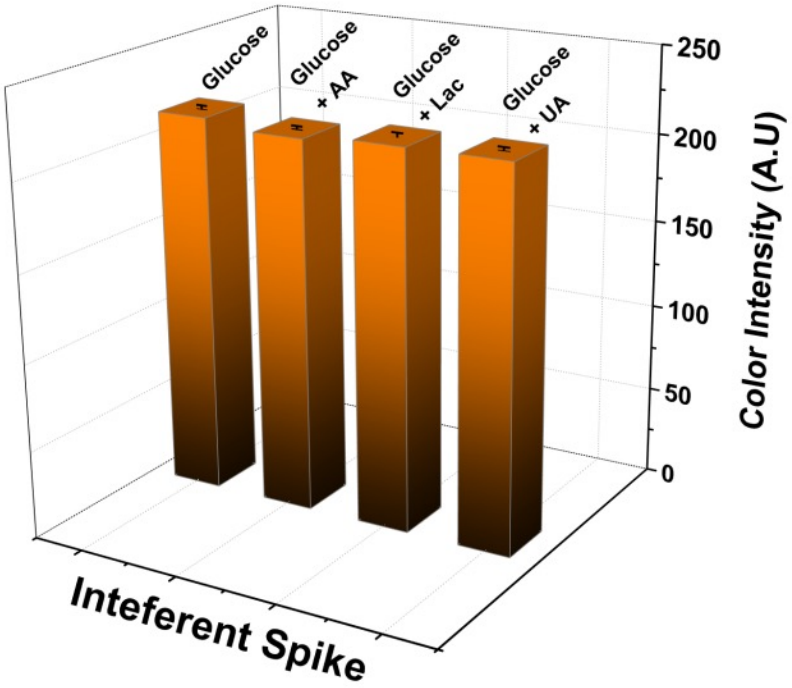

Figure S7. Interference tests for tear glucose analysis with colorimetric measurements. Tear samples were spiked with 100 µM of ascorbic acid (AA), 10 mM of lactate (Lac) and 100 µM of uric acid (UA).