Supplementary Materials for:

Paper Biosensors for Detecting Elevated IL-6 Levels in Blood and Respiratory Samples from COVID-19 patients

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Table S1: Limit of detection (LOD) and dynamic range of previously proposed biosensors for IL-6 detection

				Instrumental			
Ref	**Technique**	**Detection**	**Support**	**LOD**	**Matrix**	**Dynamic range**	**Analysis time**
1	Aptamers	Conductance	Carbon nanotube	1 pg mL^{-1}	PBS	1 pg mL^{-1} – 10 ng mL^{-1}	Real-time
2	Immunosensor and aptamer	Electrochemical	Organic Field Effect Transistors	20 pg mL^{-1}	PBS	20 pg mL^{-1} – 210 ng mL^{-1}	-
3	Immunosensor	ELISA	Ultrafiltration regenerated cellulose membranes (RC)	31 pg mL^{-1}	PBS	31 – 500 pg mL^{-1}	2 h
4	Immunosensor	Naked eye Optical spectroscopy	Magnetic nanoparticles (MNPs) and polystyrene (PS) microparticles	11 pg mL^{-1} eye 1.2 pg mL^{-1} instr	PBS	3.7 – 900 pg mL^{-1}	1 h
5	Immunosensor	Chemiluminescence	Polydimethylsiloxane (PDMS)	1.0 pg mL^{-1}	PBS (30% fetal calf serum)	5 – 1280 pg mL^{-1}	90 min
6	Immunosensor	Localized-surface plasmon resonance (LSPR)	Gold nanorod	10 pg mL^{-1}	PBS	10 – 10 000 pg mL^{-1}	30 min
7	Immunosensor	Electrochemical	Silicon nanowire field effect transistor	-	PBS	5 – 50000 pg mL^{-1}	Real-time
8	Immunosensor	Fluorescence	Optical fiber	0.1 pg mL^{-1}	PBS	0.4 – 400 pg mL^{-1}	60 min
9	Immunosensor	Electrochemical	Graphene oxides	5 pg mL^{-1}	PBS	5 − 150 pg mL^{-1}	30 min
10	Immunosensor	Electrochemical	Gold electrode	220 pg mL^{-1}	PBS	-	60 min 10 s signal
11	Immunosensor	Electrochemical	Gold electrode	4 pg mL^{-1}	PBS	-	>110 min
12	Oligonucleotides and antibodies	Optical	Silica wafers coated with Ti/Au	88 µg mL^{-1}	PBS and SSC	-	-
13	Immunosensor	Surface-enhanced Raman scattering (SERS)	Paper and DTNB on gold nano shell with a silica core	1 pg mL^{-1}	PBS	1 pg mL^{-1} – 1 µg mL^{-1}	-

[14]	Aptasensor	Electrochemical	Glassy carbon electrode modified with p-aminobenzoic acid, p-aminothiophenol and AuNPs	1.6 pg mL^{-1}	PBS	5 pg mL^{-1} – 100 ng mL^{-1}	>60 min
[14]	Immunosensor	Electrochemical	Magnetic microparticles and planar graphite-screen printed electrodes	0.3 pg mL^{-1}	PBS	1 pg mL^{-1} – 1 µg mL^{-1}	30 - 60 min
[15]	Immunosensor	Fluorescence	Nitrocellulose PVC Glass fiber	0.37 pg mL^{-1}	PBST-BSA	2 – 500 pg mL^{-1}	15 min
[16]	Immunosensor	Fluorescence spectroscopy	Nitrocellulose	0.9 pg mL^{-1}	PBS	1 – 1000 pg mL^{-1}	30 min
[17]	Aptameric GFET (field effect transistor)	Electrochemical	Graphene	210 pg mL^{-1}	PBS	1 – 16 ng mL^{-1}	6 min
[18]	Immunosensor	Differential pulse voltammetry	TI:Au on silicon subtrate	20 pg mL^{-1}	PBS	0 – 60 pg mL^{-1}	2.5 min
[19]	Immunosensor	Chemiluminescence	Optical fiber	1.05 pg mL^{-1}	PBS	5 – 10 000 pg mL^{-1}	>75 min
[20]	Immunosensor	Electrochemical	Indium tin oxide (ITO) electrode	6.0 fg mL^{-1}	PBS	0.02 – 16 pg mL^{-1}	>30 min
[21]	Immunosensor	Colorimetric	Plasmonic nanoprobes and paper	0.1 pg mL^{-1}	PBS	---	17 min
[22]	Immunosensor	Localized-surface plasmon resonance (LSPR)	Poly(pyrrole N-hydroxy succinimide)	10 fg mL^{-1}	PBS	0.03 – 22.5 pg mL^{-1}	-
[23]	Multiplexed immunosensor	LSPR	glass	11.29 pg mL^{-1}	PBS	10 – 10^4 pg mL^{-1}	40 min
[24]	Microfluidic immunoarray	electrochemical	gold	0.05 to 2 pg mL^{-1}	serum	sub pg mL−1 to well above ng mL−1)	<60 min
This work	**Immunosensor**	**Colorimetric**	**Paper/AuNPs**	**10^{-3} pg mL^{-1}**	**PBS**	**10^{-3} – 10^2 pg mL^{-1}**	**7-8 min**

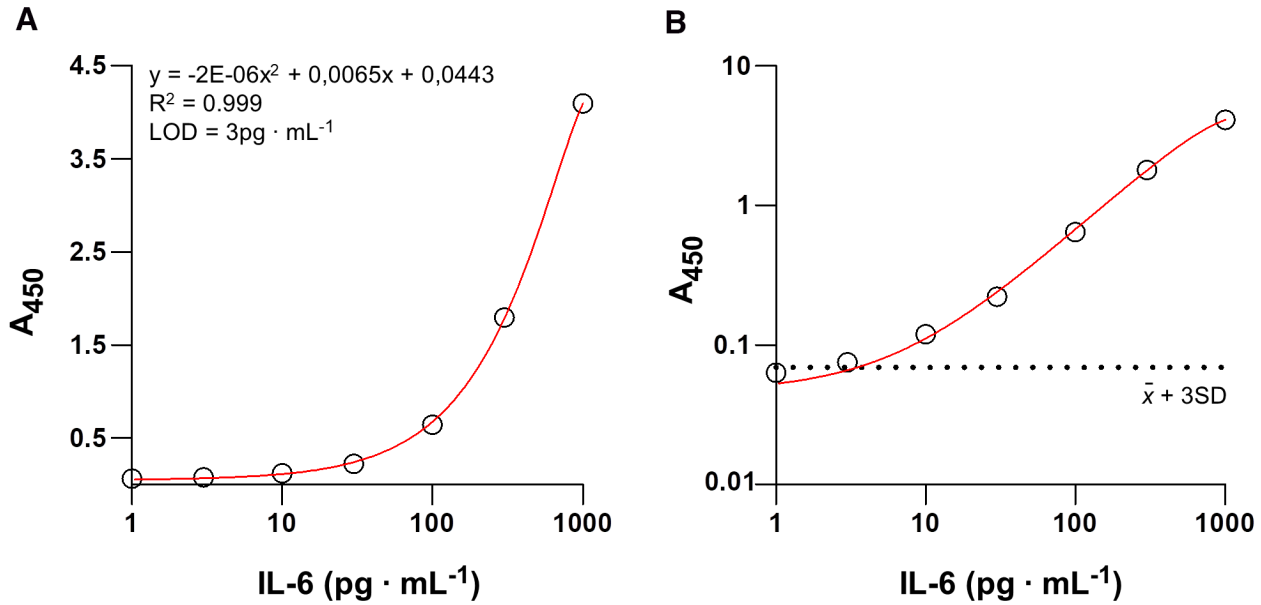

Figure S1. Calibration plot for IL-6 detection with an in-house ELISA in semi-logarithmic (A) or log-log (B) scale. The calibration plot was obtained as follows. A 96-well ELISA microplate (Thermo Scientific) was coated with 100 µl of mouse anti-human interleukin-6 (IL-6) monoclonal capture antibody (Abcam) at 1 µg·mL^{-1} in bicarbonate buffer (0.1 M, pH 9.6). After overnight incubation at 4 ºC, wells were washed 3 times with PBS containing 0.1% Tween 20 (PBST), blocked during 2 h at 37 ºC with PBS containing 2% of bovine serum albumin (BSA) and washed again 3 times with PBST. The calibration curve was obtained by adding 100 µl of IL-6 solutions to coated wells and incubated 2h at room temperature (RT) in a swinging shaker. IL-6 solutions were also applied to wells without capture antibodies in order to subtract non-specific signals. Each sample was assayed in triplicate. Next, plates were washed 3 times with PBST and then 100 µL of biotinylated mouse anti-human IL-6 monoclonal detection antibody (Sigma-Aldrich) at 10 µg·mL^{-1} in PBST-BSA 1% was added. After 2h of incubation at RT in a swinging shaker, plates were washed 5 times with PBST and 100 µL of streptavidin-HRP diluted 1:1000 in 1% PBST-BSA was added for 30 min at RT. Then, plates were washed again 5 times with PBST and 100 µL of ready-to-use 1-Step Ultra TMB (Thermo Scientific) was added for

30 min at RT. Finally, the colorimetric reaction was stopped with 100 µL of 2N H_2SO_4, and absorbance was measured at 450 nm. Absorbance was read with a Biotek power wave plate reader. The limit of detection expressed as the sample that yields a signal above three times the standard deviation of the blank is 3 pg·mL^{-1}.

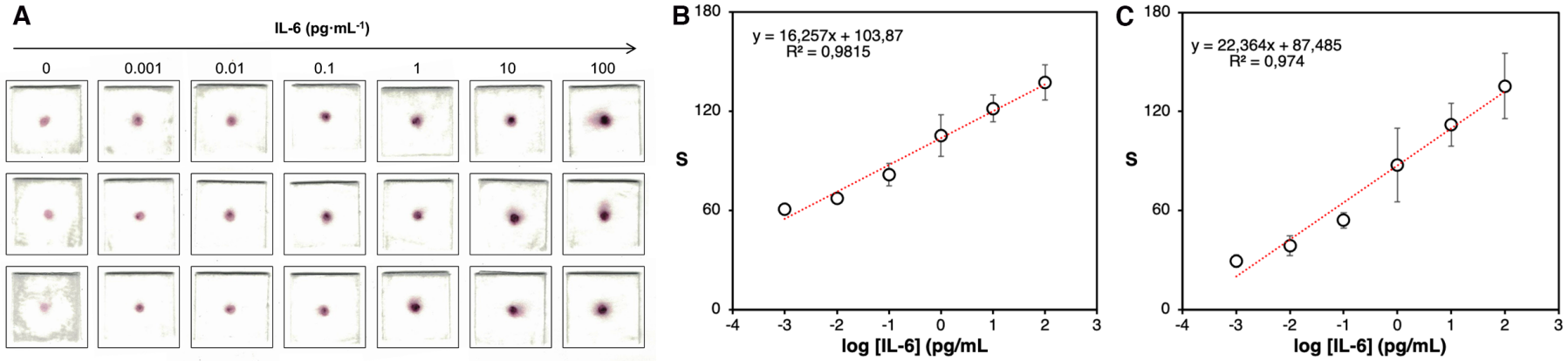

Figure S2. Calibration plot with IL-6 diluted in PBS-BSA (40 µg mL^{-1}) instead of in PBS. (**A**) Scanned images of the paper biosensors; (**B**) Calibration plot obtained via densitometric analysis of images in (A) with ImageJ; $S_{blank} + 3\sigma = 59$; (C) Calibration plot obtained when measuring the colorimetric signal of the same assays with our app; $S_{blank} + 3\sigma = 23$. Error bars are the standard deviation of the three independent experiments.

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