Supporting Information

A DNA biosensors-based microfluidic platform for attomolar real-time detection of unamplified SARS-CoV-2 virus

Perrine Robin[a, 1], Laura Barnabei[b,1], Stefano Marocco[c,1], Jacopo Pagnoncelli[c], Daniele Nicolis[f], Chiara Tarantelli[b], Agatino Christian Tavilla[f], Roberto Robortella[f], Luciano Cascione[b], Lucas Mayoraz[a], Céline M. A. Journot[a], Mounir Mensi[d], Francesco Bertoni[b,e,*], Igor Stefanini[c,f,*], Sandrine Gerber-Lemaire[a,*]

[1] Contributed equally to the work

[a] Group for Functionalized Biomaterials, Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland
[b] Institute of Oncology Research, Faculty of Biomedical Sciences, USI, Via Francesco Chiesa 5, CH-6500 Bellinzona, Switzerland
[c] Medical Devices area, Institute of Digital Technologies for Personalized Healthcare - MeDiTech, Department of Innovative Technologies, University of Applied Sciences of Southern Switzerland, Via la Santa 1, CH-6962 Lugano – Viganello, Switzerland
[d] ISIC-XRDSAP, EPFL Valais-Wallis, Rue de l'Industrie 17, CH-1951 Sion, Switzerland
[e] Oncology Institute of Southern Switzerland, Ente Ospedaliero Cantonale, CH-6500 Bellinzona, Switzerland
[f] Department of Innovative Technologies, University of Applied Sciences of Southern Switzerland, Via la Santa 1, CH-6962 Lugano – Viganello, Switzerland

* Corresponding authors.
francesco.bertoni@ior.usi.ch; +41 58 666 72 06 (F. Bertoni)
igor.stefanini@supsi.ch; +41 58 666 66 75 (I. Stefanini)
sandrine.gerber@epfl.ch; +41 21 693 93 72 (S. Gerber-Lemaire)

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Specificity of PP1 for SARS-CoV-2 virus

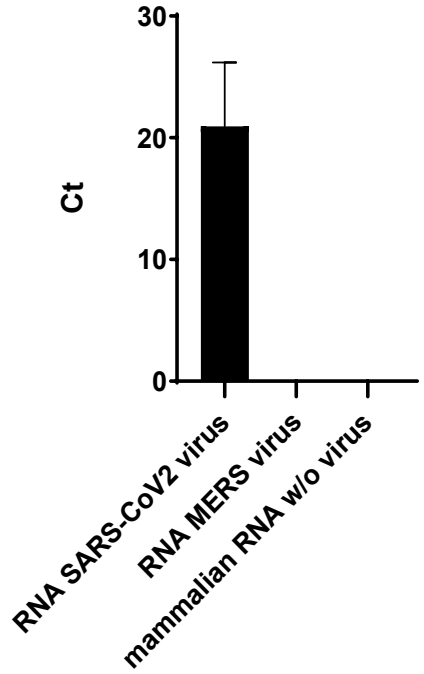


Figure S1. Specific amplification of RNA from SARS-CoV-2 positive sample and not from SARS-CoV-2 negative samples. The primers used to perform the qPCR were: 5' TGAAGCAAGGTGAAATCAAGGA 3' and 5'AACAGCAAGAAGTGCAACGCCAAC 3' (as probe **PP1**).

Quantification of immobilized ssDNA probe on functionalized SiO_2 slides

Slide	**S-PP1**	**S-Suc** (negative control)
DNA amount quantified (per face)	2.90 pmol 1.94 pmol 2.46 pmol 3.57 pmol	0.11 pmol 0.21 pmol 0.00 pmol
Average	2.7 ± 0.7 pmol	0.10 ± 0.10 pmol

Table S1. Quantity of surface-immobilized ssDNA probe on **S-PP1** and **S-Suc** slides.

Name	**Sequence**
PP1	5'AmMC6-AACAGCAAGAAGTGCAACGCCAAC
Cy3-comp. PP1	5'Cy3-GTTGGCGTTGCACTTCTTGCTGTT

Table S2. DNA sequences corresponding to PP1 and 5'-Cy3-labelled complementary DNA strand.

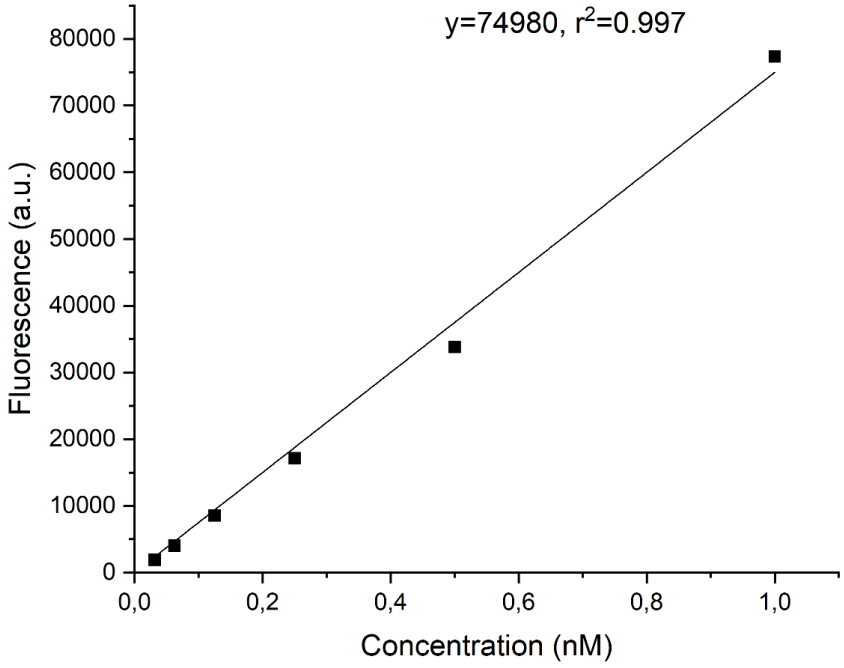


Figure S2. Representative standard curve for the quantification of Cy3-complementary **PP1** strand liberated from a surface.

Characterization of functionalized SiO_2 slides by XPS

Slides	Si at%	C at%	N at%	P at%
S-NH_2	42.48	49.15	8.37	0.00
S-Suc	39.32	52.62	8.06	0.00
S-PP1	38.77	54.08	6.86	0.30

Table S3. Surface relative atomic concentration of C, N, O and S detected via XPS for amino-modified slides **S-NH_2**, succinic-modified slides **S-Suc** and DNA-functionalized slides **S-PP1**.

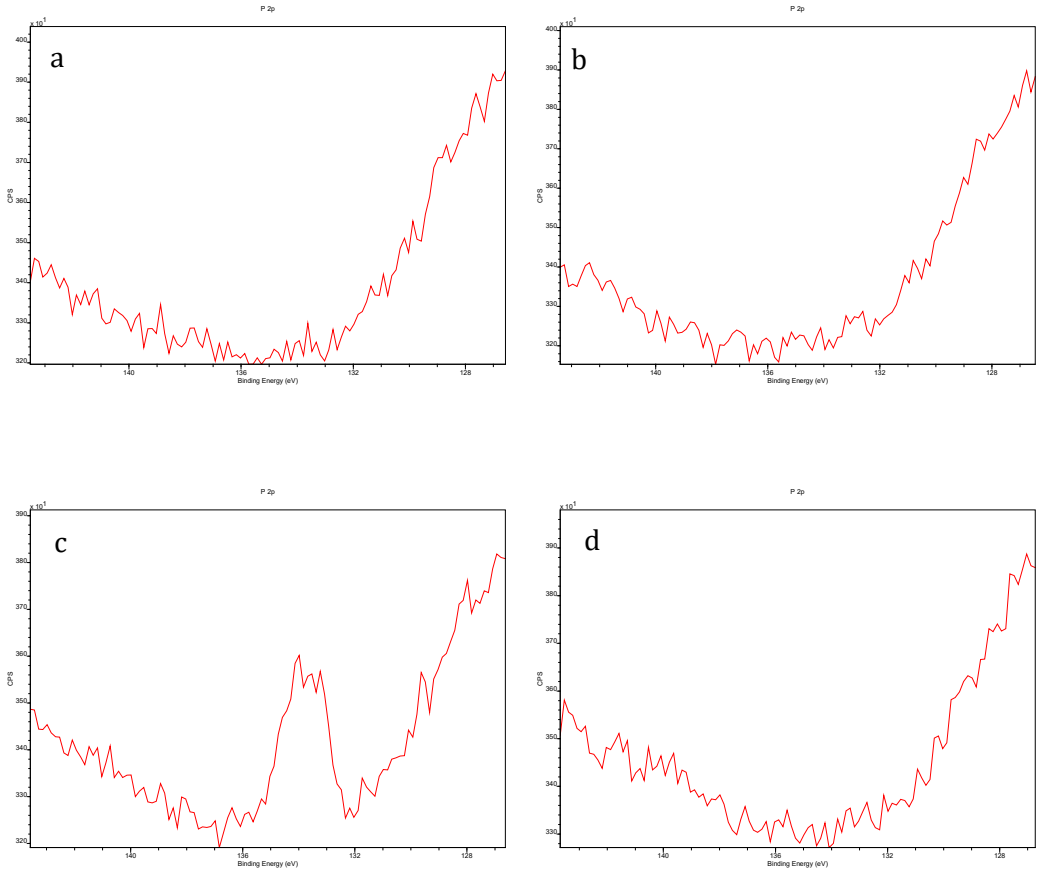


Figure S3. XPS spectra of P 2p region of (a) amino-modified slides **S-NH_2**; (b) **S-Suc** slides; (c) DNA-functionalized slides **S-PP1**; (d) **S-Suc** slides incubated with **PP1** probes without coupling agent.

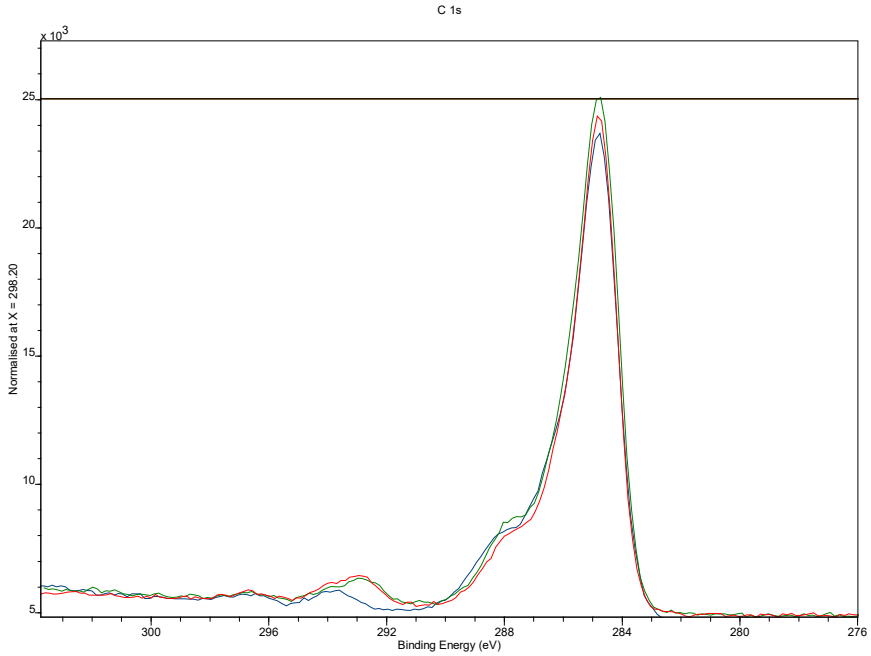


Figure S4. XPS spectra of C 1s region of amino-modified slides **S-NH$_2$** (red), succinic-modified slides **S-Suc** (green) and DNA-functionalized slides **S-PP1** (blue). Normalized at 298.20eV.

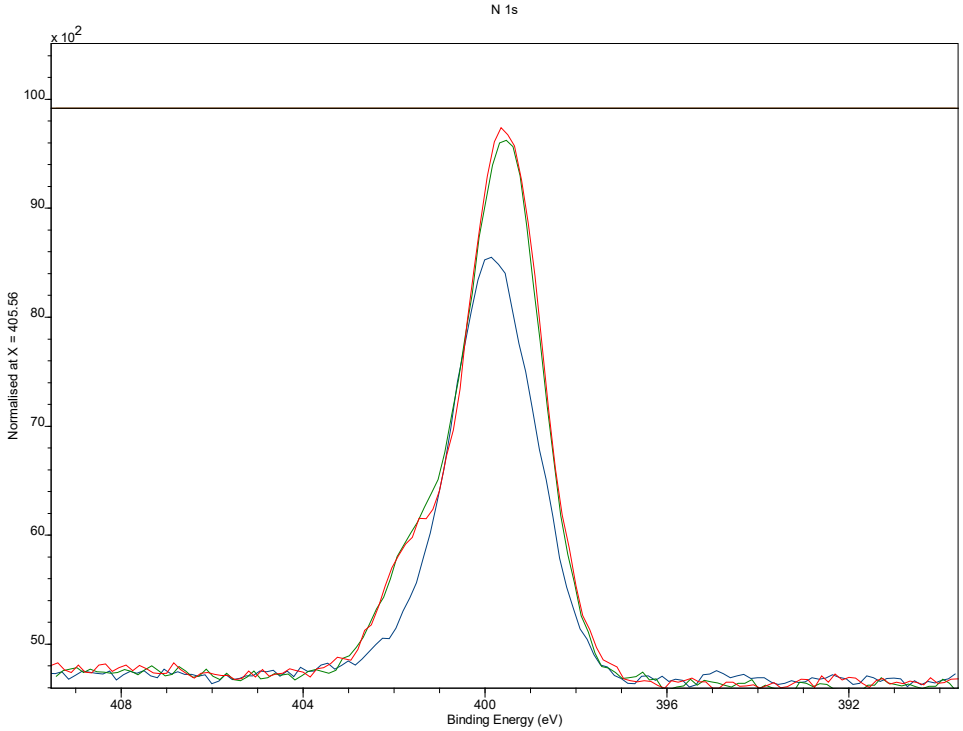


Figure S5. XPS spectra of N 1s region of amino-modified slides **S-NH$_2$** (red), succinic-modified slides **S-Suc** (green) and DNA-functionalized slides **S-PP1** (blue). Normalized at 405.56eV

Assessment of the stability of S-PP1 slides

Storage conditions	SSC 4X	MiliQ	Tris.HCl 1M	PBS 1X	Dried without solvent	Dried with solvents
DNA density (pmol/cm^2) After 10 days	3.07 ± 0.3	2.25 ± 0.78	3.89 ± 0.43	3.00 ± 0.53	2.52 ± 0.13	1.50 ± 0.2
DNA density (pmol/cm^2) After 15 days	/	2.55 ± 0.71	/	/	/	/
DNA density (pmol/cm^2) After 20 days	/	2.63 ± 0.62	/	/	/	/

Table S4. Quantification of surface immobilized **PP1** DNA probe on **S-PP1** slides after 10 days of storage at 4°C in different media. Quantification of surface immobilized **PP1** DNA probe on **S-PP1** slides after 15 and 20 days of storage at 4°C in MilliQ. Values are expressed as mean ± standard deviation (triplicate).

Conception diagram of the automated SARS-CoV-2 diagnostics platform

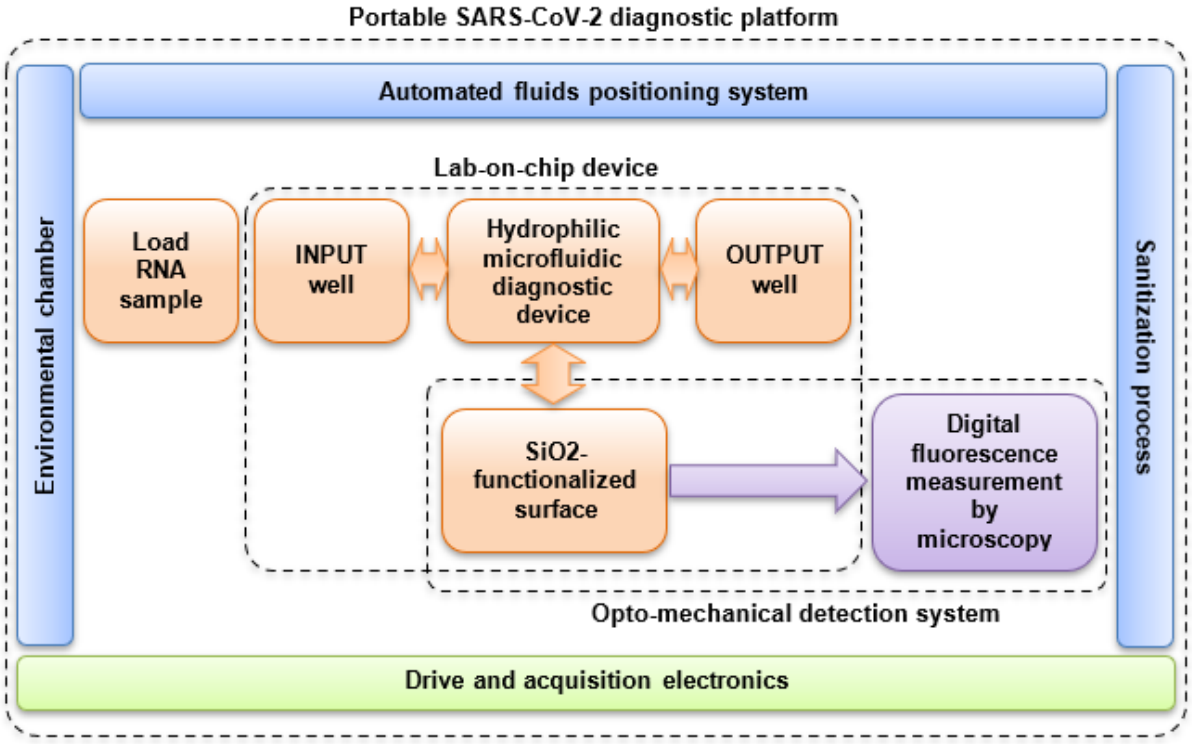


Figure S6. Main block diagram of the automatic device for viral screening via DNA-based biosensors.

Comparison of RNA extraction protocols

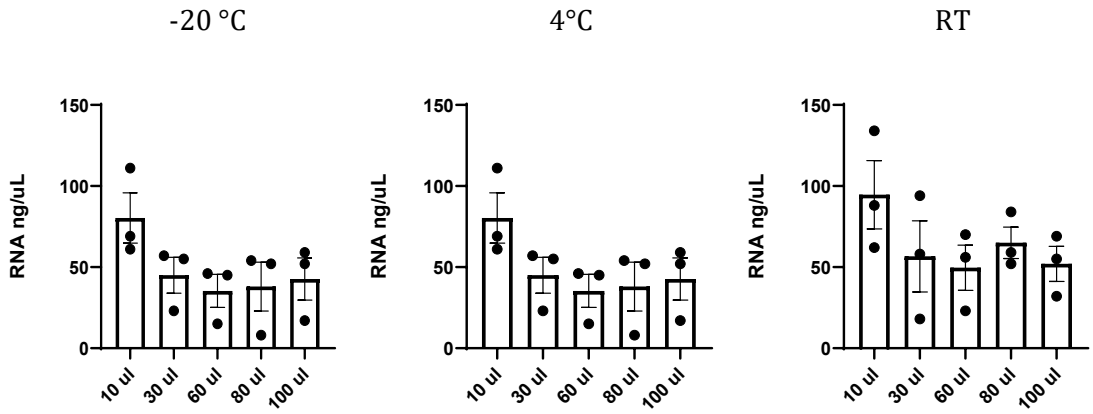


Figure S7. RNA extraction performed using different concentration of human saliva mixed with Lucigen Quick Extract DNA kit (1:1). No significative difference was observed keeping the kit at -20°C (as manufacturer's instruction), 4°C or RT.

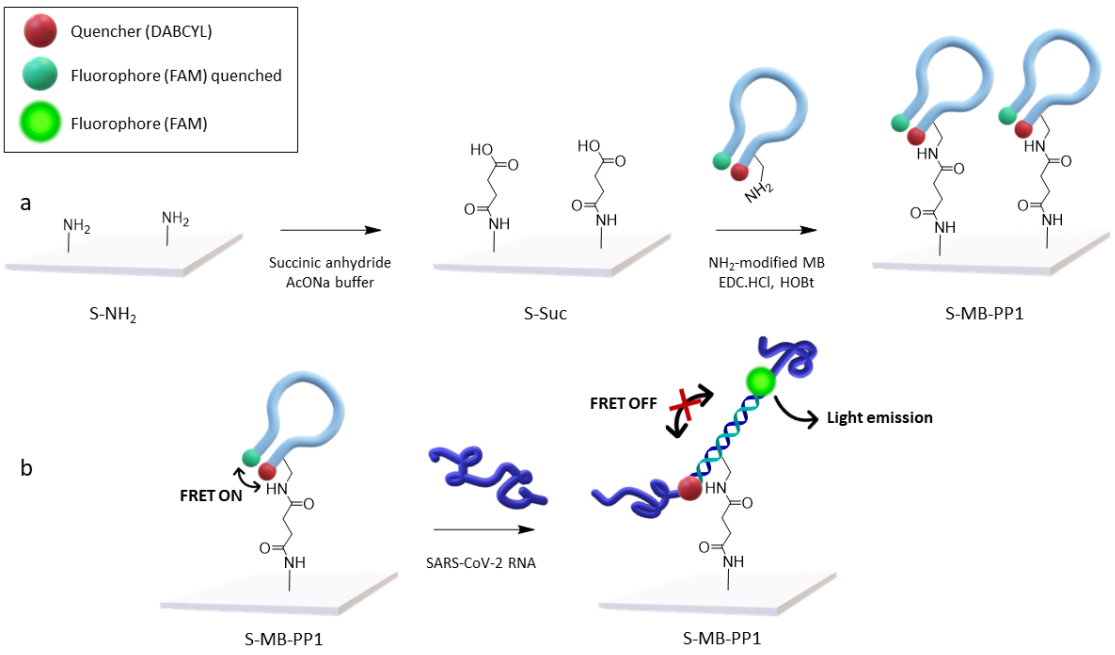
Evaluation of sensing slides functionalized with the molecular beacon MB-PP1 probe

Name	**Sequence**
MB-PP1	5'AmMC6-TT TTT TTT TTT TT-FAM- TGA CGG AAC AGC AAG AAG TGC AAC GCC AAC CCG TCA-DABCYL-3'

Table S5. DNA sequence corresponding to **MB-PP1**. DABCYL: N-[4-(4-dimethylamino)phenylazo]benzoic acid; FAM: Carboxyfluorescein. The FAM fluorophore was internally integrated to the probe through click chemistry.

Slide	**S-MB-PP1**	**S-Suc** (negative control)
DNA amount quantified (per face)	5.42 pmol 3.67 pmol	0.73 pmol 0.00 pmol
Average	4.5 ± 0.9 pmol	0.37 ± 0.52 pmol

Table S6. Quantity of surface-immobilized **MB-PP1** probes on **S-MB-PP1** and **S-Suc** slides



Scheme S1. a: Conjugation of **MB-PP1** probes to the surface of sensing slides. b: Principle of SARS-CoV-2 RNA detection with **S-MB-PP1** slides. DABCYL: N-[4-(4-dimethylamino)phenylazo]benzoic acid; FAM: Carboxyfluorescein; FRET: Fluorescence Resonance Energy Transfer.

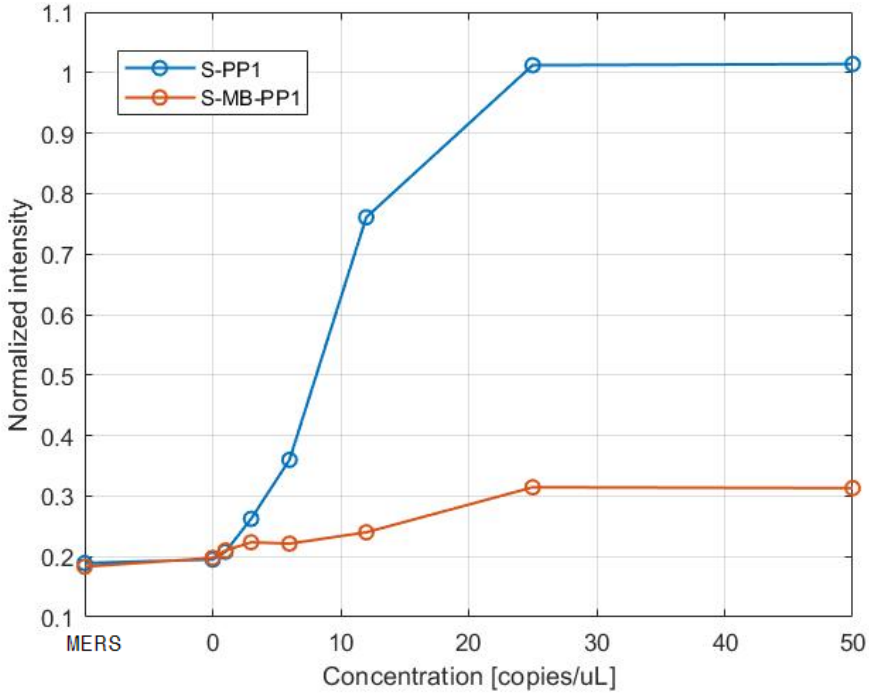


Figure S8. Comparison of the sensing properties of **S-PP1** and **S-MB-PP1** slides. Light intensities emitted by SYBR Green I (**S-PP1** samples) and carboxyfluorescein (**S-MB-PP1** samples) were converted by the PMT and normalized by subtraction of baseline values (**S-PP1** slides with SYBR Green I only or **S-MB-PP1** slides). **S-PP1** and **S-MB-PP1** were incubated with saliva samples containing SARS-CoV-2 virus at concentrations of 50, 25, 12, 6, 3, 1 copies/µL or MERS virus at a concentration of 50 copies/µL.

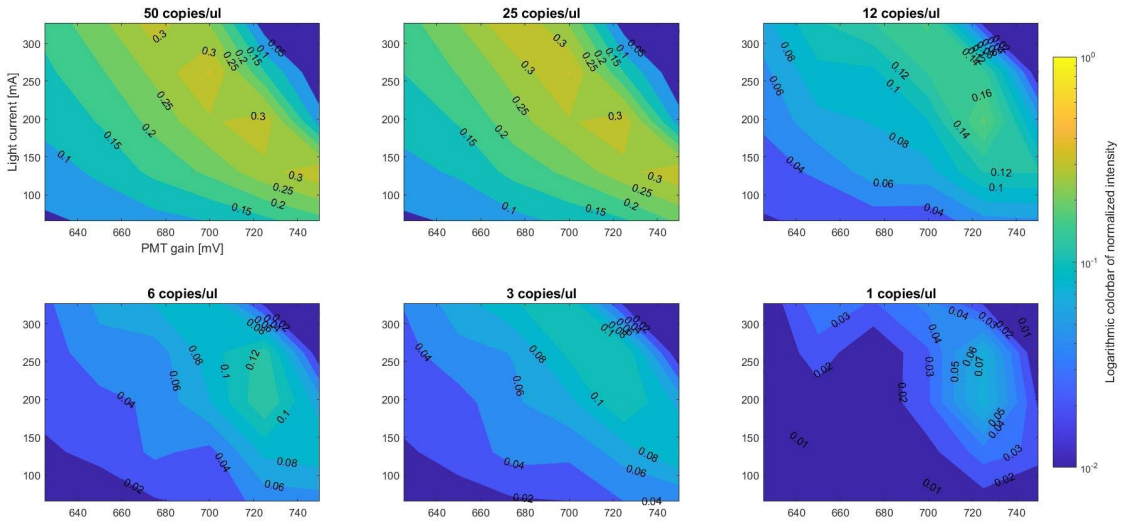


Figure S9. Difference between **S-MB-PP1** samples and baseline at decreasing SARS-CoV-2 viral loads (50, 25, 12, 6, 3, 1 copies/µL) represented as logarithmic colorbars of normalized intensities over LED current intensity (mA) and PMT gain (mV).