Electronic Supplementary Information

Nanotechnology Meets Immunology Towards a Rapid Diagnosis Solution: The COVID-19 Outbreak Challenge

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Evaluation of AuNP, antibodies concentration, and AuNP:HAb ratio.

The overall features of both bare and conjugate nanoparticles depend on the concentration of the metallic *nuclei* and the amount of HAb located on their surface. The estimation of both [BIOCOR_VID19_30] and BIOCOR_VID19_30:HAb (AuNP:HAb ratio) ratio was performed using the initial concentration of the precursors, the atomic mass and density of Au (MM_{Au} = 197 g/mol; ρ_{Au}= 19.3 g/cm^3), and the results of the mean diameter of the AuNP obtained along the synthesis steps. So, DLS measurements were performed following several synthesis stages, and the results are summarized in Table S1.

Table S1 Summary of results based on DLS data for initial, partials, and final steps of the synthesis of BIOCOR_VID19_30.

Synthesis step	Au^{3+} accumulated volume (mL)	Au^{3+} accumulated quantity (mmol)	Mean H_D (nm)	Polydispersity Index (PDI)	Volume of each AuNP (nm^3)
1	1.0	0.01	12	0.12	905
2	2.0	0.02	18	0.13	3054
5	5.0	0.05	26	0.08	9203
7	7.0	0.07	30	0.07	14138

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For the calculations, the following assumptions were applied:

i. The nanoparticles can be considered perfect spheres.

ii. The total number of nanoparticles must be formed during the first step of the synthesis - nuclei formation, and the following steps must collaborate only for nanoparticle growth.

iii. Considering the initial amount of Au^{3+} (0.010 mmol) and the obtained diameter of *nuclei* generated in the first step of the synthesis (2R=12 nm), the theoretical value of [AuNP], considering 100% of reaction yield, is [AuNP] = 0.70 μM.

During conjugation, it was used 0.875 mL of AuNP suspension in a final volume of 1 mL leading to a concentration of [AuNP] = 0.61 μM and the concentration of [HAb] in the final volume was set to 100 μg/mL (0.66 μM). Then, the molar ratio AuNP:HAb ~1:1.1.

After conjugation, for LFA assays, the volume of suspension with the conjugate AuNP:antibody was reduced by half, increasing by 2 times the final concentration at conjugation step.

Table S2 Bioconjugated Nanomaterials for the Diagnosis of the SARS-CoV-2 Antigen or Virus (PRAMANIK et al., 2022, copyright – ACS on 28-sept-2022. DOI: 10.1021/accountsmr.1c00177)

target molecules	detection platform	bioconjugated nanostructure used	detection time	detection sensitivity	ref
SARS-CoV-2 N gene	colorimetric	RNA/DNA-attached gold nanoparticle	10 min	0.18 ng/μL	16
SARS-CoV-2 spike protein	colorimetric	antibody-conjugated gold nanoparticles	5 min	1 ng/mL	23
SARS-CoV-2 spike protein	colorimetric	MNAzyme-conjugated gold nanoparticles	5 min	90% for clinical samples	39
SARS-CoV-2 S, N & P gene	colorimetric	antibody-conjugated gold nanoparticles	20 min	95% for clinical samples	21
SARS-CoV-2 IgM/IgG antibodies	colorimetric-based lateral flow	nucleoprotein-attached gold nanoparticles	15 min	93% for clinical samples	27
SARS-CoV-2 RNA	colorimetric CRISPR/Cas	RNA/DNA-attached gold nanoparticle	15 min	95.2% for clinical samples	22
SARS-CoV-2 spike protein	SERS	antibody-conjugated gold nanoparticles	10 min	4 pg/mL	23
SARS-CoV-2 spike protein	SERS	antibody-attached graphene	15 min	3.75 fg/mL	38
SARS-CoV-2 IgM/IgG antibodies	SERS-based LFA	antigen-attached SiO_2@Ag nanoparticle	20 min	100% for clinical samples	35
SARS-CoV-2 spike protein	SERS	antibody-conjugated gold nanoparticles	20 min	87.7% clinical sample	42
SARS-CoV-2 spike protein	NSET	aptamer-conjugated gold nanoparticles	10 min	130 fg/mL	24
SARS-CoV-2 spike protein	fluorescence	ACE2-attached SWCNT	90 min	12.6 nM	37
SARS-CoV-2 IgG and IgM	fluorescence-based LFA	antigen/SiO_2@Au@QD nanobeads	15 min	100% for clinical samples	28
SARS-CoV-2 spike protein	NSET	antibody-coated quantum dots	20 min	200 fg/mL	30
SARS-CoV-2 spike protein	field effect transistor	antibody-coated MXene-graphene	50 s	1 fg/mL	36
SARS-CoV-2 RNA	RT-PCR		6 h	97% for clinical samples	8
SARS-CoV-2 RNA	CRISPR-Cas		40 min	95% for clinical samples	11
SARS-CoV-2 antigen	LFA		15 min	95% for clinical samples	8

Figures

A)

		not infected				infectede				P2 reactivity test and J4 and J5 peptides				
		1	2	3	4	5	6	7	8	9	10	11	12	
J4	A	C1	C1	C2	C2	C1	C1	C2	C2	+	+	-	-	Samples
J5	B	C1	C1	C2	C2	C1	C1	C2	C2	+	+	-	-	
P2	C	C1	C1	C2	C2	C1	C1	C2	C2					
J4	D	+	+	-	-	+	+	-	-	+	+	-	-	controls
J5	E	+	+	-	-	+	+	-	-	+	+	-	-	
P2	F	+	+	-	-	+	+	-	-	+	+	-	-	
	G													
	H													

Controls (lines D-E-F):
Column 1 a 4: PBS+serum+conjugate
Column 5 a 8: PBS+serum
Column 9 a 12: peptídeo+serum

B)

C)

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0,05	0,048	0,049	0,05	0,058	0,058	0,058	0,061	0,145	0,204	0,697	0,604	490
B	0,046	0,046	0,048	0,047	0,166	0,465	0,239	0,435	0,211	0,13	0,625	0,382	490
C	0,612	0,641	0,464	0,588	0,659	0,62	0,633	0,857	0,052	0,054	0,053	0,051	490
D	0,048	0,049	0,047	0,047	0,05	0,049	0,045	0,046	0,056	0,062	0,054	0,055	490
E	0,05	0,047	0,047	0,047	0,06	0,055	0,045	0,048	0,214	0,363	0,046	0,049	490
F	0,063	0,057	0,049	0,064	0,072	0,054	0,053	0,056	1,147	1,045	0,629	0,517	490
G	0,037	0,037	0,036	0,038	0,039	0,036	0,037	0,037	0,038	0,041	0,036	0,037	490
H	0,037	0,039	0,037	0,037	0,037	0,036	0,037	0,037	0,04	0,059	0,049	0,041	490

Figure S1 A) cross-reaction ELISA plate map. B) finished ELISA plate. C) Absorbance readings measured in the spectrophotometer after completion of the ELISA.

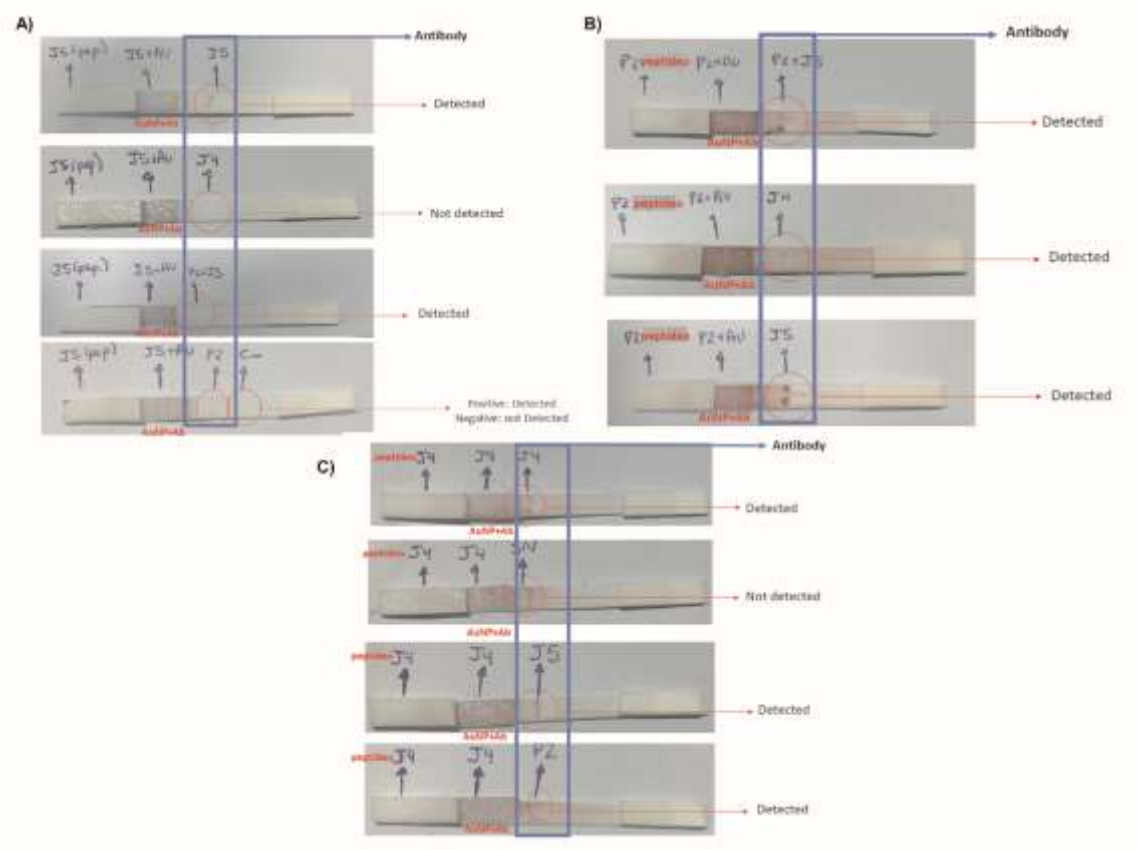

Figure S2 A) LFA sample: peptide J5; AuNP: peptide J5; membrane: anti-peptide J5 – anti-peptide J4 – anti-peptide P2+J5 and anti-peptide P2+negative sera respectively; B) LFA sample: peptide P2; AuNP: peptide P2; membrane: anti-peptide P2+J5 – anti-peptide J4 and anti-peptide J5 respectively; C) LFA sample: peptide J4; AuNP: peptide J4; membrane: anti-peptide J4 – Negative sera – anti-peptide J5 and anti-peptide P2 respectively.

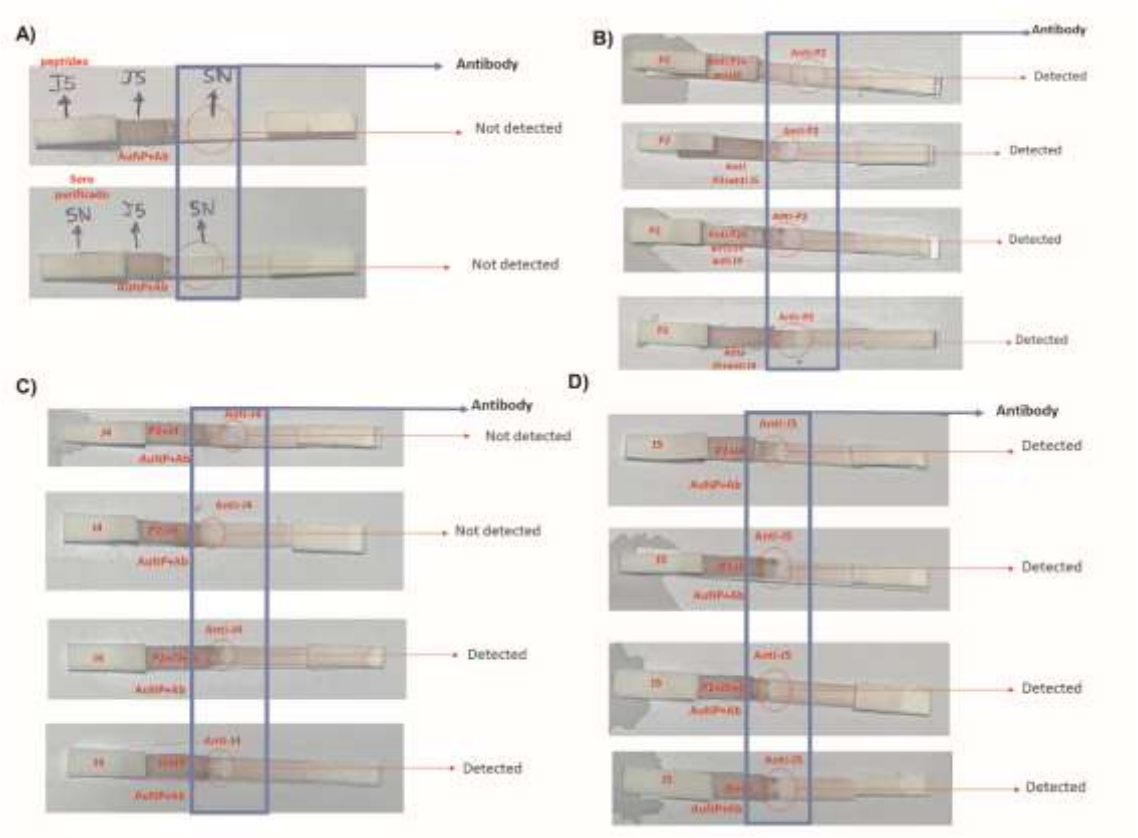

Figure S3 A) LFA sample: peptide J5 and negative sera respectively; AuNP: and anti-peptide J5; membrane: negative sample; B) LFA sample: peptide P2; AuNP: anti-peptide P2+anti-peptide J4 - anti-peptide P2+anti-peptide J5 - anti-peptide P2+anti-peptide J4+ anti-peptide J5 and anti-peptide J5+anti-peptide J4 respectively; membrane: anti-peptide P2; C) LFA sample: peptide J4; AuNP: anti-peptide P2+anti-peptide J4 - anti-peptide P2+anti-peptide J5 - anti-peptide P2+anti-peptide J4+ anti-peptide J5 and anti-peptide J5+anti-peptide J4 respectively; membrane: anti-peptide J4; D) LFA sample: peptide J5; AuNP: anti-peptide P2+anti-peptide J4 - anti-peptide P2+anti-peptide J5 - anti-peptide P2+anti-peptide J4+ anti-peptide J5 and anti-peptide J5+anti-peptide J4 respectively; membrane: anti-peptide J5.

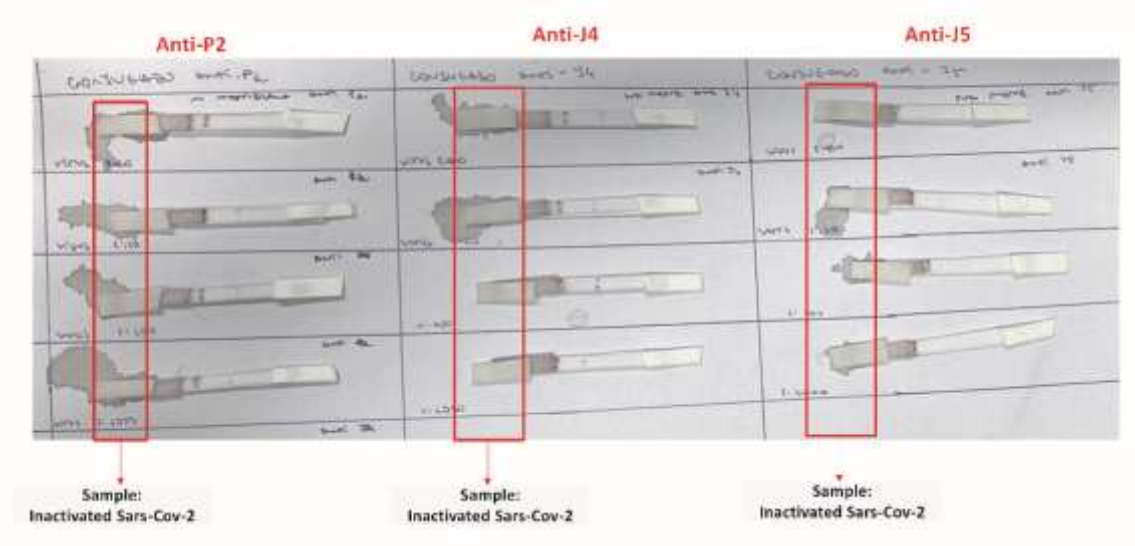

Figure S4 Anti-P2: LFA sample: SARS-CoV-2 inactivated pure and in the dilutions 1:10, 1:100, and 1:1000 respectively; AuNP: anti-peptide P2; membrane: anti-peptide P2. **Anti-J4**: LFA sample: SARS-CoV-2 inactivated pure and in the dilutions 1:10, 1:100, and 1:1000, respectively; AuNP: anti-peptide P2; membrane: anti-peptide J4. **Anti-J5**: LFA sample: SARS-CoV-2 inactivated pure and in the dilutions 1:10, 1:100, and 1:1000, respectively; AuNP: anti-peptide P2; membrane: anti-peptide J5.