



## Article

# Monoclonal Antibodies against Nucleocapsid Protein of SARS-CoV-2 Variants for Detection of COVID-19

Ruei-Min Lu <sup>1,†</sup>, Shih-Han Ko <sup>1,†</sup>, Wan-Yu Chen <sup>2</sup>, Yu-Ling Chang <sup>1</sup>, Hsiu-Ting Lin <sup>2</sup> and Han-Chung Wu <sup>1,2,\*</sup><sup>1</sup> Biomedical Translation Research Center (BioTReC), Academia Sinica, Taipei 11529, Taiwan; reminlu@gate.sinica.edu.tw (R.-M.L.); shko@gate.sinica.edu.tw (S.-H.K.); a29259963@hotmail.com (Y.-L.C.)<sup>2</sup> Institute of Cellular and Organismic Biology, Academia Sinica, Taipei 11529, Taiwan; sirirem59@gmail.com (W.-Y.C.); 3772.monico@gmail.com (H.-T.L.)

\* Correspondence: hcw0928@gate.sinica.edu.tw

† These authors contributed equally.

## Supplementary materials

**Table S1.** Prototype lateral flow immunoassays using different antibody pairs.

mAb pair	Capture mAb	7															39					
		7	39	40	42	46	49	51	52	53	7	39	40	42	46	49	51	52	53			
Antigen	NP from <i>E. coli</i>	0.1	0.5	0.5	0	0	0	0	0	0.5	1.5	0	1	0	0	0	0	0	0	0	0	2
	NP from 293 cells	1	0	1	0	0	0	0	0.1	1.5	0.5	0	0	0	0	0	0	0	0	0	0	0.5
	<b>Sum of rating</b>	1.1	0.5	1.5	0	0	0	0	0.1	2	2	0	1	0	0	0	0	0	0	0	0	2.5
	Capture mAb																					42
mAb pair	Detection mAb	7	39	40	42	46	49	51	52	53	7	39	40	42	46	49	51	52	53			
		NP from <i>E. coli</i>	1	0.5	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Antigen	NP from 293T cells	1.5	0	0	0	0	0	0	0	1	0.5	0	0	0	0	0	0	0	0	0	0	0
	<b>Sum of rating</b>	2.5	0.5	0	0	0	0	0	0	2	0.5	0	0	0	0	0	0	0	0	0	0	0
mAb pair	Capture mAb																					49
		7	39	40	42	46	49	51	52	53	7	39	40	42	46	49	51	52	53			
Antigen	NP from <i>E. coli</i>	0	0	0.5	0	0	0	0	0	0.5	0.1	0.5	0.5	0	0	0	0	0	0	0	0	0.5
	NP from 293T cells	0.5	0	0	0	0	0	0	0	0.1	1	0	0.5	0	0	0	0	0	0	0	0	0.5
	<b>Sum of rating</b>	0.5	0	0.5	0	0	0	0	0	0.6	1.1	0.5	1	0	0	0	0	0	0	0	0	1
mAb pair	Detection mAb																					52
		7	39	40	42	46	49	51	52	53	7	39	40	42	46	49	51	52	53			
Antigen	NP from <i>E. coli</i>	0	0	0	0	0	0	0	0	0	0.1	0	0.5	0	0	0	0	0	0	0	0	1.5
	NP from 293T cells	0.5	0	0	0	0	0	0	0	0.1	1.5	0	1	0	0	0	0	0	0	0	0	1
	<b>Sum of rating</b>	0.5	0	0	0	0	0	0	0	0.1	1.6	0	1.5	0	0	0	0	0	0	0	0	2.5
mAb pair	Capture mAb																					53
		7	39	40	42	46	49	51	52	53												-
Antigen	NP from <i>E. coli</i>	1	2	0.5	0	0	0	0	0	0.5	0											
	NP from 293T cells	2	0	1	0	0	0	0	0	0	0											
	<b>Sum of rating</b>	3	2	1.5	0	0	0	0	0	0.5	0											

Note: One hundred microliters of 100 ng/mL recombinant NP purified from *E. coli* and 293T cells was used for each antigen sample. Rating chart (intensity range from 0 to 5) shows grading of the positive and negative results. Latex color intensity equal or greater than 0.5 was considered a positive specimen (high positive: ≥ 3; medium positive ≥ 2; low positive ≥ 1); less than 0.5 was considered negative. Results were interpreted within 10 min of sample application.

**Table S2.** The list of human common respiratory pathogens of cross-reactivity study.

Bacteria Panel	Bacteria Counts (CFU/mL)	Negative Group (without SARS-CoV-2)			Positive Group (with SARS-CoV-2)		
<i>Bordetella pertussis</i>	$1 \times 10^6$	–	–	–	+	+	+
<i>Chlamydia pneumoniae</i>	$1 \times 10^6$	–	–	–	+	+	+
<i>Escherichia coli</i>	$2 \times 10^8$	–	–	–	+	+	+
<i>Haemophilus influenzae</i>	$3 \times 10^8$	–	–	–	+	+	+
<i>Mycoplasma pneumoniae</i>	$1 \times 10^6$	–	–	–	+	+	+
<i>Pseudomonas aeruginosa</i>	$3 \times 10^8$	–	–	–	+	+	+
<i>Staphylococcus aureus</i>	$7 \times 10^8$	–	–	–	+	+	+
<i>Staphylococcus epidermidis</i>	$6 \times 10^8$	–	–	–	+	+	+
<i>Streptococcus pneumoniae</i>	$7 \times 10^8$	–	–	–	+	+	+
<i>Streptococcus pyogenes</i>	$1 \times 10^6$	–	–	–	+	+	+
Viral Panel	Virus Titer	Negative Group (without SARS-CoV-2)			Positive Group (with SARS-CoV-2)		
<b>Adenovirus type 7</b>	$2.81 \times 10^5$ TCID <sub>50</sub> /mL	–	–	–	+	+	+
<b>Human coronavirus 229E</b>	$6 \times 10^5$ PFU/mL	–	–	–	+	+	+
<b>Human coronavirus OC43</b>	$1 \times 10^5$ PFU/mL	–	–	–	+	+	+
<b>Enterovirus type 68</b>	$8.91 \times 10^5$ TCID <sub>50</sub> /ml	–	–	–	+	+	+
<b>Enterovirus type 71</b>	$2.11 \times 10^7$ TCID <sub>50</sub> /mL	–	–	–	+	+	+
<b>Human Parainfluenza Virus (HPIV)</b>	$1.19 \times 10^5$ TCID <sub>50</sub> /mL	–	–	–	+	+	+
<b>Influenza A -H1N1</b>	$1.7 \times 10^5$ TCID <sub>50</sub> /mL	–	–	–	+	+	+
<b>Influenza A -H3N2</b>	$1.8 \times 10^5$ TCID <sub>50</sub> /mL	–	–	–	+	+	+
<b>Influenza B -Vic</b>	$9.2 \times 10^5$ TCID <sub>50</sub> /mL	–	–	–	+	+	+
<b>Influenza B -Yam</b>	$4 \times 10^5$ TCID <sub>50</sub> /mL	–	–	–	+	+	+
<b>Respiratory syncytial virus type B</b>	$1 \times 10^5$ PFU/mL	–	–	–	+	+	+
<b>Rhinovirus</b>	$6.5 \times 10^6$ TCID <sub>50</sub> /mL	–	–	–	+	+	+

Note: CFU: Colony-Forming Units; PFU: Plaque-Forming Units; TCID<sub>50</sub>: 50% of Tissue Culture Infectious Dose; -: Negative result; +: Positive result.

**Table S3.** Materials used in interference assays.

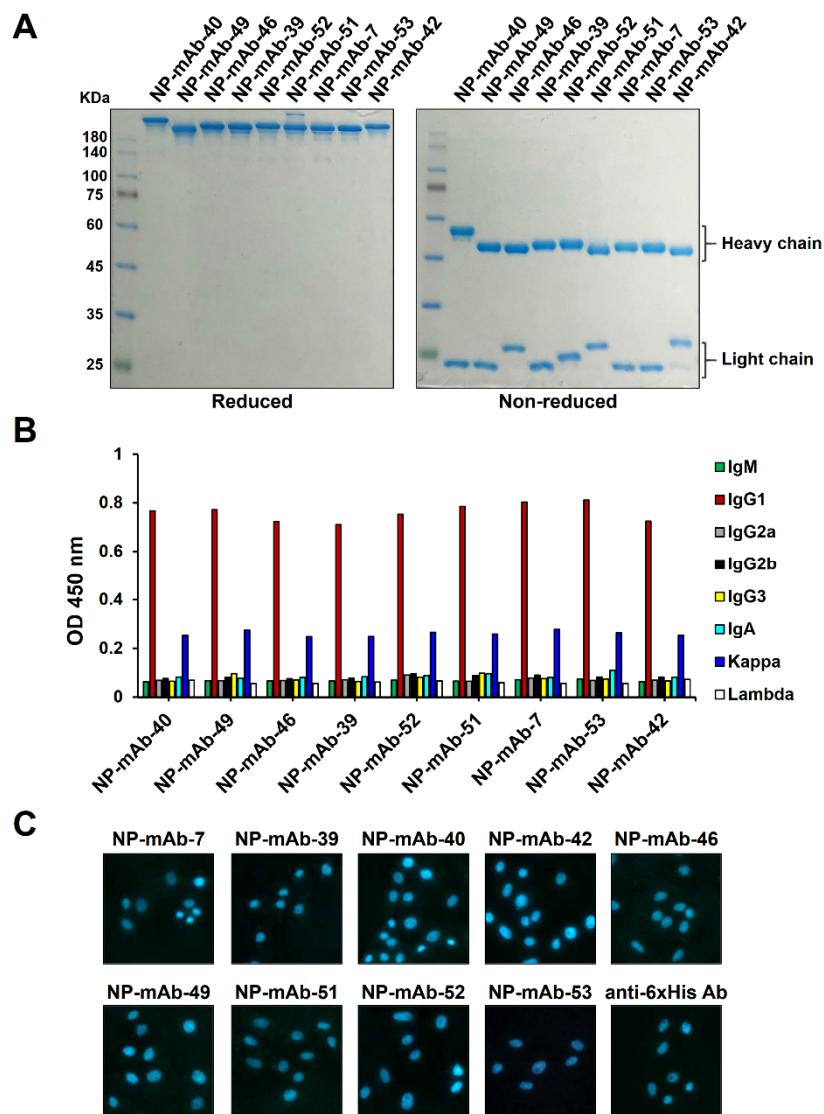
Interfering Substances	Concentration	Negative Group (without SARS-CoV-2)			Positive Group (with SARS-CoV-2)		
Mucin	4%	–	–	–	+	+	+
Whole blood	5%	–	–	–	+	+	+
Aspirin	20 mg/mL	–	–	–	+	+	+
Dextromethorphan	10 mg/mL	–	–	–	+	+	+
Diphenhydramine HCl	5 mg/mL	–	–	–	+	+	+
Hemoglobin	20 mg/mL	–	–	–	+	+	+
Hosoon Troches (ROOT)	20 mg/mL	–	–	–	+	+	+
Nasal Washing Salt	20 mg/mL	–	–	–	+	+	+
Nasal Ointment	10%	–	–	–	+	+	+
NASONEX Aqueous Nasal Spray	10%	–	–	–	+	+	+
Oxymetazoline HCl	10 mg/mL	–	–	–	+	+	+
Phenylephrine HCl	100 mg/mL	–	–	–	+	+	+
Postan	20 mg/mL	–	–	–	+	+	+
Swinin nasal sprays	10%	–	–	–	+	+	+
Ibuprofen	20 mg/mL	–	–	–	+	+	+

Note: – : Negative result; + : Positive result.

**Table S4.** Comparison of COVID-19 NP antigen LFIA tests.

Product Name	Company/Sponsor	LoD (TCID <sub>50</sub> /mL)	Accuracy Information	Type of Sample	Approved
BinaxNOW COVID-19 Ag Card	Abbott Diagnostics Scarborough, Inc.	140.6	Sensitivity: 84.6% Specificity: 98.5%	NS	US EUA
SARS-CoV-2 Rapid Antigen Test	Roche	494	Sensitivity: 95.5% Specificity: 99.2%	NP	CE marking
NP-mAb-40/7 LFIA (Acadecise)	Academia Sinica, Taipei, Taiwan	547	Sensitivity: ≥ 99% Specificity: ≥ 99%	NP	TFDA EUA
Sienna-Clarity COVID-19 Antigen Rapid Test Cassette	Salofa Oy	1250	Sensitivity: 87.5% Specificity: 98.9%	NP	US EUA
SPERA COVID-19 Ag Test	Ttrava Health	1560	Sensitivity: 91.8% Specificity: 96.9%	NS	US EUA
STANDARD Q COVID-19	SD-Biosensor	1981	Sensitivity: 96.5% Specificity: 99.7%	NP	CE marking
InteliSwab COVID-19 Rapid Test Pro	OraSure Technologies, Inc.	2500	Sensitivity: 84% Specificity: 98%	NS	US EUA

Note: LoD, limit of detection; NS, nasal swab; NP, nasopharyngeal swab; EUA, Emergency Use Authorization; TFDA, Taiwan Food and Drug Administration. Arranged in LoD.



**Figure S1.** Characterization of NP-specific antibodies. (A) Nine antibodies were produced in ascites and purified by protein G sepharose. SDS-PAGE and Coomassie blue staining were conducted to identify heavy chain and light chain of each antibody. (B) Nine anti-NP antibodies were isotype with ELISA-based isotyping kits. (C) Immunofluorescence staining corresponds to Figure 1E. Mock Vero cells were probed with 1  $\mu$ g/mL anti-NP mAb, followed by FITC-goat-anti-mouse IgG. Anti-6xHis Ab with rhodamine-conjugated secondary antibody was used to confirm NP expression.