Certificate of Analysis for NR-56461

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/MD-HP20874/2021 (Lineage B.1.1.529; Omicron Variant)

Catalog No. NR-56461

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/MD-HP20874/2021 was isolated from a nasal swab in Maryland, USA, on November 27, 2021. NR-56461 lot 70049434 was produced by infecting *Homo sapiens* lung adenocarcinoma epithelial cells (Calu-3; ATCC® HTB-55™) with the deposited material and incubating in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 7 days at 37°C with 5% CO_2. The cells and supernatant were spin-clarified at 1500 × g for 10 minutes at 4°C.

Passage History:

V/T/A(1)/C(1) (Johns Hopkins University/BEI Resources); V/T/A= *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 gene (TMPRSS2) and angiotensin-converting enzyme 2 (ACE2); C = Calu-3

Lot: 70049434 Manufacturing Date: 17DEC2021

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TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Calu-3 Cells	Cell rounding and detachment	Cell rounding and detachment
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform (Refer to Appendix I for NGS information)	≥ 98% identity with SARS-CoV-2, hCoV-19/USA/MD-HP20874/2021 (GISAID: EPI_ISL_7160424)	99.99% identity with SARS-CoV-2, hCoV-19/USA/MD-HP20874/2021 (GISAID: EPI_ISL_7160424)
Titer by $TCID_{50}$ Assay in Calu-3 Cells by Cytopathic Effect[1] (8 days at 37°C and 5% CO_2)	Report results	4.4×10^5 $TCID_{50}$ per mL[2]
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic[3]	No growth	No growth
Trypticase Soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C, aerobic	No growth	No growth
Mycoplasma Contamination		
Agar and broth culture (14-day incubation at 37°C)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

[1]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.

[2]Titer was determined by cytopathic effects (CPE) and completed in triplicate (8.9×10^5 per mL, 2.8×10^5 per mL and 1.6×10^5 per mL). The average of the three values is reported.

[3]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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Program Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

Support Provided by NIAID

APPENDIX I: NGS Information for NR-56461 lot 70049434

The sequence submission in GISAID for omicron isolate hCoV-19/USA/MD-HP20874/2021 is EPI_ISL_7160424. This reference contains many stretches of Ns, some of which covered regions that included lineage-defining mutations. To search for a better reference the original reference was submitted to GISAID's Audacity Instant tool, which finds the most similar sequences to given query sequence. The most similar sequence, EPI_ISL_7605630, was nearly identical to the provided reference except for two differences: 1) EPI_ISL_7605630 doesn't include the first 8, non-N nts found in the original reference, which occur well ahead of ORF1ab and 2) EPI_ISL_7605630 has no N regions. EPI_ISL_7605630 was therefore chosen as the reference for analysis for comparing BEI produced virus stocks because its lack of Ns allowed for the detection of all the lineage-defining mutations.

Sequence analysis using SBC v2.0 pipeline and LoFreq version 2.1.5 and freebayes version: v1.3.1 variant callers resulted in the discovery of one SNP when compared to the reference sequence from GISAID EPI_ISL_7605630 (see Table I below). Additionally, both the reference sequence EPI_ISL_7605630 and NR-56461 lot 70049434 contained fifty-five SNPs, seven deletions (DEL) and one insertion (INS) when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table II below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Table I: Variants with different nucleotides between NR-56461 lot 70049434 and reference sequence EPI_ISL_7605630

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	a18298g	2060	1	6.3107%	ORF1ab (nsp14)	I87V

Table II: Variants with different nucleotides between NR-56461 lot 70049434 and GenBank MN908947.3 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)

Variant Type	Variant Position and Identified Alternative Base	Coverage[1]	Length of Variant	Frequency of Variant[1]	Gene (Region)	Amino Acid Mutation
SNP	c241t	n/a	1	100.0000%	5'UTR	Untranslated
SNP	a2832g	n/a	1	100.0000%	ORF1ab (nsp3)	K38R
SNP	c3037t	n/a	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	t5386g	n/a	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c5730t	n/a	1	100.0000%	ORF1ab (nsp3)	T1004I
DEL	Δ6513-6515	n/a	-3	100.0000%	ORF1ab (nsp3)	S1265I, ΔL1266
SNP	g8393a	n/a	1	100.0000%	ORF1ab (nsp3)	A1892T
SNP	c10029t	n/a	1	100.0000%	ORF1ab (nsp4)	T492I
SNP	c10449a	n/a	1	100.0000%	ORF1ab (nsp5)	P132H
DEL	Δ11283-11291	n/a	-9	100.0000%	ORF1ab (nsp6)	ΔLSG (amino acid 105-107)
SNP	a11537g	n/a	1	100.0000%	ORF1ab (nsp6)	I189V
SNP	t13195c	n/a	1	100.0000%	ORF1ab (nsp10)	Silent mutation

Variant Type	Variant Position and Identified Alternative Base	Coverage[1]	Length of Variant	Frequency of Variant[1]	Gene (Region)	Amino Acid Mutation
SNP	c14408t	n/a	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	c15240t	n/a	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	a18163g	n/a	1	100.0000%	ORF1ab (nsp14)	I42V
SNP	c21762t	n/a	1	100.0000%	Spike	A67V
DEL	Δ21765-21770	n/a	-6	100.0000%	Spike	ΔHV (amino acid 69-70)
SNP	c21846t	n/a	1	100.0000%	Spike	T95I
DEL	Δ21987-21995	n/a	-9	100.0000%	Spike	G142D, ΔVYY (amino acid 143-145)
DEL	Δ22194-22196	n/a	-3	100.0000%	Spike	N211I, ΔL212
INS	22204[gagccagaa] 22205	n/a	+9	100.0000%	Spike	214[EPE]215
SNP	g22578a	n/a	1	100.0000%	Spike	G339D
SNP	t22673c	n/a	1	100.0000%	Spike	S371L
SNP	c22674t	n/a	1	100.0000%		
SNP	t22679c	n/a	1	100.0000%	Spike	S373P
SNP	c22686t	n/a	1	100.0000%	Spike	S375F
SNP	g22813t	n/a	1	100.0000%	Spike	K417N
SNP	t22882g	n/a	1	100.0000%	Spike	N440K
SNP	g22898a	n/a	1	100.0000%	Spike	G446S
SNP	g22992a	n/a	1	100.0000%	Spike	S477N
SNP	c22995a	n/a	1	100.0000%	Spike	T478K
SNP	a23013c	n/a	1	100.0000%	Spike	E484A
SNP	a23040g	n/a	1	100.0000%	Spike	Q493R
SNP	g23048a	n/a	1	100.0000%	Spike	G496S
SNP	a23055g	n/a	1	100.0000%	Spike	Q498R
SNP	a23063t	n/a	1	100.0000%	Spike	N501Y
SNP	t23075c	n/a	1	100.0000%	Spike	Y505H
SNP	c23202a	n/a	1	100.0000%	Spike	T547K
SNP	a23403g	n/a	1	100.0000%	Spike	D614G
SNP	c23525t	n/a	1	100.0000%	Spike	H655Y
SNP	t23599g	n/a	1	100.0000%	Spike	N679K
SNP	c23604a	n/a	1	100.0000%	Spike	P681H
SNP	c23854a	n/a	1	100.0000%	Spike	N764K
SNP	g23948t	n/a	1	100.0000%	Spike	D796Y
SNP	c24130a	n/a	1	100.0000%	Spike	N856K
SNP	a24424t	n/a	1	100.0000%	Spike	Q954H
SNP	t24469a	n/a	1	100.0000%	Spike	N969K
SNP	c24503t	n/a	1	100.0000%	Spike	L981F
SNP	c25000t	n/a	1	100.0000%	Spike	Silent mutation
SNP	c25584t	n/a	1	100.0000%	ORF3a	Silent mutation

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Variant Type	Variant Position and Identified Alternative Base	Coverage[1]	Length of Variant	Frequency of Variant[1]	Gene (Region)	Amino Acid Mutation
SNP	c26270t	n/a	1	100.0000%	Envelope	T9I
SNP	a26530g	n/a	1	100.0000%	Membrane	D3G
SNP	c26577g	n/a	1	100.0000%	Membrane	Q19E
SNP	g26709a	n/a	1	100.0000%	Membrane	A63T
SNP	a27259c	n/a	1	100.0000%	ORF6	Silent mutation
SNP	c27807t	n/a	1	100.0000%	ORF7b	Silent mutation
SNP	a28271t	n/a	1	100.0000%	Intergenic (ORF8/ Nucleocapsid)	Untranslated
SNP	c28311t	n/a	1	100.0000%	Nucleocapsid	P13L
DEL	Δ28362-28370	n/a	-9	100.0000%	Nucleocapsid	ΔERS (amino acid 31-33)
SNP	g28881a	n/a	1	100.0000%	Nucleocapsid	R203K
SNP	g28882a	n/a	1	100.0000%		
SNP	g28883c	n/a	1	100.0000%	Nucleocapsid	G204R

[1]Coverage for all variants in Table II is listed as 'N/A'. There is no read coverage information for these variants because the sample reads are only mapped to the reference sequence and not to the SARS-CoV-2, Wuhan-Hu-1 isolate sequence (GenBank MN908947), but that does not mean these areas lack for coverage. All variants in Table II are mismatches in between the reference sequence and the SARS-CoV-2, Wuhan-Hu-1 sequence, so these variants will be assigned a frequency of 100% and will therefore be majority SNPs.