Certificate of Analysis for NR-54971

SARS-Related Coronavirus 2, Isolate hCoV-19/England/204820464/2020 in Calu-3 (Lineage B.1.1.7; Alpha Variant)

Catalog No. NR-54971

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/England/204820464/2020 was isolated from a human on November 24, 2020, in England, United Kingdom. NR-54971 lot 70041937 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 4 days at 37°C with 5% CO_2.

Passage History:

V-hSLAM(2)/C(1) (Public Health England/BEI Resources); V-hSLAM = Vero-hSLAM; C = Calu-3

Lot: 70041937 Manufacturing Date: 31JAN2021

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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/England/ 204820464/2020 (GISAID: EPI_ISL_683466)	99.97% identity with SARS-COV-2, hCoV-19/England/ 204820464/2020 (GISAID: EPI_ISL_683466)
Titer by $TCID_{50}$ Assay in Calu-3 Cells by Cytopathic Effect[1] (5 days at 37°C and 5% CO_2)	Report results	2.8×10^5 $TCID_{50}$ per mL
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic[2]	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]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Heather Couch/
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APPENDIX I: NGS Information for NR-54971 lot 70041937

Sequence analysis using AMGP readsQC-illumina.py and LoFreq version 2.1.5 variant caller resulted in the discovery of ten SNPs when compared to the reference sequence from GISAID EPI_ISL_683466 (see Table I below). Additionally, both the reference sequence EPI_ISL_683466 and NR-54971 lot 70041937 contained thirty-two SNPs and three deletions (DEL) 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-54971 lot 70041937 and reference sequence EPI_ISL_683466

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	g521t	1456	1	7.2802%	ORF1ab (nsp1)	V86F
SNP	t1963a	952	1	5.0420%	ORF1ab (nsp2)	Silent mutation
SNP	t1963c	952	1	6.0924%	ORF1ab (nsp2)	Silent mutation
SNP	g11873a	1400	1	11.1429%	ORF1ab (nsp7)	V11I
SNP	t13339g	2119	1	9.2496%	ORF1ab (nsp10)	N105K
SNP	t14679c	1008	1	6.0516%	ORF1ab (nsp12)	Silent mutation
SNP	t17886c	1613	1	5.2077%	ORF1ab (nsp13)	Silent mutation
SNP	t22114c	729	1	12.3457%	Spike	Silent mutation
SNP	c26542t	809	1	7.6638%	Membrane	T71I
SNP	a29839g	730	1	5.2055%	3'UTR	Untranslated

Table II: Variants with different nucleotides between NR-54971 lot 70041937 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	c913t	n/a	1	100.0000%	ORF1ab (nsp2)	Silent mutation
SNP	c3037t	n/a	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c3267t	n/a	1	100.0000%	ORF1ab (nsp3)	T183I
SNP	c5388a	n/a	1	100.0000%	ORF1ab (nsp3)	A890D
SNP	c5986t	n/a	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c6633t	n/a	1	100.0000%	ORF1ab (nsp3)	A1305V
SNP	t9654c	n/a	1	100.0000%	ORF1ab (nsp3)	I1412T
DEL	Δ11288-11296	n/a	-9	100.0000%	ORF1ab (nsp6)	ΔSGF (amino acid 106-108)
SNP	c14408t	n/a	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	c14676t	n/a	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	c15279t	n/a	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	t16176c	n/a	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	a17615g	n/a	1	100.0000%	ORF1ab (nsp13)	K460R
SNP	a19079g	n/a	1	100.0000%	ORF1ab (nsp14)	E347G
SNP	c19845t	n/a	1	100.0000%	ORF1ab (nsp15)	Silent mutation
DEL	Δ21765-21770	n/a	-6	100.0000%	Spike	ΔHV (amino acid 69-70)

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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
DEL	Δ22191-22193	n/a	-3	100.0000%	Spike	ΔY144
SNP	a23063t	n/a	1	100.0000%	Spike	N501Y
SNP	c23721a	n/a	1	100.0000%	Spike	A570D
SNP	a23403g	n/a	1	100.0000%	Spike	D614G
SNP	c23604a	n/a	1	100.0000%	Spike	P681H
SNP	c23709t	n/a	1	100.0000%	Spike	T716I
SNP	t24506g	n/a	1	100.0000%	Spike	S982A
SNP	g24914c	n/a	1	100.0000%	Spike	D1118H
SNP	c27972t	n/a	1	100.0000%	ORF8	Q27STOP
SNP	g28048t	n/a	1	100.0000%	ORF8	R521I
SNP	a28111g	n/a	1	100.0000%	ORF8	Y73C
SNP	g28280c	n/a	1	100.0000%	Nucleocapsid	D3L
SNP	a28281t	n/a	1	100.0000%		
SNP	t28282a	n/a	1	100.0000%		
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
SNP	c28977t	n/a	1	100.0000%	Nucleocapsid	S235F

[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.

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