



Figure S1 Western blot of spike and nucleocapsid peptides utilizing anti-Xpress antibody (N is nucleocapsid, S is spike, E is for elution fraction from nickel bead affinity column).

				S ELISA Epitope			
				AA617		AA649	
				612		657	
A	QQQ37295	USA/WA1/2020		YQDVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			No change = 282/291
	OQ423331.1	hCov-19/France2022		YQGVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			
	EPI_SL_16429136	SD 2022		YQGVNCTEVPVAIADQLTPTWRVYSTG <b>E</b> NVFQTRAGCLIGAEHVN			S640F = 5/291
	UKO09277.1	PA 2021		YQGVNCTE <b>I</b> PVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			V620I = 1/291
	EPI_SL_9347718	PA 2021		YQGVNCTEVPVAIADQLTPTWRVYSTG <b>G</b> NVFQTRAGCLIGAEHVN			S640C = 1/291
	EPI_SL_16429123	KS 2022		YQGVNCTE <b>V</b> SAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			P621S = 1/291
	EPI_SL_16300816	PA 2022		YQGVNCTEVPVAIADQLTPTWRVYSTG <b>S</b> VFQTRAGCLIGAEHVN			N641S = 1/291
	EPI_SL_4847030	OH 2021		YQGVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			
	EPI_SL_17810978	NJ 2022		YQGVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			
	EPI_SL_17810977	NJ 2022		YQGVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			
	EPI_SL_16467176	KY 2022		YQGVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			
	EPI_SL_16372324	IL 2022		YQGVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			
	EPI_SL_16464978	MA 2021		YQGVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			
	EPI_SL_16429147	OK 2022		YQGVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			
	EPI_SL_16297218	NY 2021		YQGVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			
	EPI_SL_16297277	NJ 2021		YQGVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			
	EPI_SL_16297280	NC 2021		YQGVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			
	EPI_SL_13610682	NY 2021		YQGVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			
	EPI_SL_16429133	TN 2022		YQGVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			
	EPI_SL_16366919	NC 2021		YQGVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			
	EPI_SL_16297266	LA 2021		YQGVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			
	EPI_SL_17810984	KS 2021		YQGVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			
	EPI_SL_17810979	TN 2022		YQGVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			
	EPI_SL_17810980	MN 2021		YQGVNCTEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			
	EPI_SL_17810976	NJ 2022		YQGVN <b>C</b> TEVPVAIADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN			T618I = 1/291
	Consensus			*** ** * : *****			
				N ELISA Epitope			
				AA360		AA416	
B	QQQ37295	USA/WA1/2020		YKTFPPTEPKDKKKKKADETQALPQRQKKQQTVTLLPAADLDDFSKQLQQSMSSADS			No change = 155/291
	OQ423331.1	hCov-19/France2022		YKTFPPTEPKDKKKKKADETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			
	EPI_SL_16467175	WV 2022		YKTFPPTEPKDKKKKKADETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			
	EPI_SL_16388471	WV 2022		YKTFPPTEPKDK <b>K</b> KKKADETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			K373N = 5/291
	EPI_SL_16366932	NY 2022		YKTFPPTEPKDKKKKKADETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSR <b>V</b> DS			A414V = 1/291
	EPI_SL_4847033	OH 2021		YKTFPPTEPKDKKKKKADETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSR <b>A</b> D			S416L = 1/291
	EPI_SL_5804769	IA 2020		YKTFPPTEPKDK <b>K</b> KAYETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			K373R, D377Y = 1/291
	EPI_SL_16297298	MA 2022		YKTFPPTEPKDKKKKKAYETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMS <b>T</b> ADS			D377Y, S413I = 1/291
	EPI_SL_16388465	TN 2022		YKTFPPTEPKDKKKKKAYETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSR <b>A</b> D			D377Y, S416L = 1/291
	EPI_SL_16372324	IL 2022		YKTFPPTEPKDKKKKKAYETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			
	EPI_SL_16464978	MA 2021		YKTFPPTEPKDKKKKKAYETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			
	EPI_SL_16429147	OK 2022		YKTFPPTEPKDKKKKKAYETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			
	EPI_SL_16297218	NY 2021		YKTFPPTEPKDKKKKKAYETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			
	EPI_SL_16297277	NJ 2021		YKTFPPTEPKDKKKKKAYETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			
	EPI_SL_16297280	NC 2021		YKTFPPTEPKDKKKKKAYETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			
	EPI_SL_13610682	NY 2021		YKTFPPTEPKDKKKKKAYETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			D377Y = 124/291
	EPI_SL_16429136	SD 2022		YKTFPPTEPKDKKKKKAYETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			
	EPI_SL_16429133	TN 2022		YKTFPPTEPKDKKKKKAYETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			
	EPI_SL_16366919	NC 2021		YKTFPPTEPKDKKKKKAYETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			
	EPI_SL_16297266	LA 2021		YKTFPPTEPKDKKKKKAYETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			
	EPI_SL_17810984	KS 2021		YKTFPPTEPKDKKKKKAYETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			
	EPI_SL_17810979	TN 2022		YKTFPPTEPKDKKKKKAYETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			
	EPI_SL_17810980	MN 2021		YKTFPPTEPKDKKKKKAYETQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			
	EPI_SL_16297242	MA 2021		YKTFPPTEPKDKKKKKAY <b>E</b> IQALPQRQKKQQTVTLLPAADLDDFSKQLQQMSRADS			T379I = 1/291
	Consensus			***** ** *			

Figure S2 Sequence alignment of spike and nucleocapsid epitope sequences. (A) Spike ELISA epitope designed based upon the sequence of USA/Wa1/2020 aligned hCoV-19France 2022 and deer sample amino acids sequences generated from GISAID repository. (B) Nucleocapsid ELISA epitope designed based upon the sequence of USA/Wa1/2020 aligned hCoV-19France 2022 and deer sample amino acids sequences generated from GISAID repository Columns from left to right are sequence identifier (either NCBI or GISAID) strain name or United States state from which sequence originated, year sequence was obtained, amino acid sequence, specific amino acid substitution identified, and prevalence among 291 full-length, high-coverage deer sequences deposited in GISAID. Asterisks denote conserved amino acid; colon denotes conserved amino acid change.

NCBI Identifier	Clade, Lineage	AA331	AA413	AA445	AA454	AA478	AA484	AA536
QOQ37295	USA/WA/2020	198.A						
UQL70209.1		20C.B.1						
UQL70221.1		20C.B.1						
UQL70233.1		20G.B.1						
UQL70244.1		20G.B.1.1.2						
UQL70255.1	Ohio	20G.B.1.2						
UQL70266.1		20G.B.1						
UQL70277.1		20G.B.1						
UQL70288.1		20G.B.1						
UQL70299.1		20G.B.1						
UQL70310.1		20G.B.1						
UK005044.1		20C.B.1.2						
UGS92736.1		20G.B.1.2						
UER37262.1	New York	20A.B.1						
UK009289.1		21J.AY.103						
UK009311.1	Pennsylvania	21J.AY.88						
UK009242.1		21J.AY.103						
UK009277.1		21J.AY.103						
Consensus								

Figure S3 Clustal Omega alignment of S domain amino acids 331 through 536 of select deer isolates from 2020/2021. Amino acids changes from original USA/WA/2020 strain are depicted with yellow highlighting. Asterisks are identical sequences; colons are conservative amino acid changes, empty spaces are unconserved amino acid substitutions.

