

Supplementary Material

Pre-existing Cross-reactive Immunity to Highly Pathogenic Avian Influenza 2.3.4.4b A(H5N1)

Virus in the United States

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Supplementary Table S1. Antigens used in the multiplex influenza antibody detection (MIADA) assay

Assays (N-plex)	Antigen group	Antigen	Virus strain	Type (subtype or lineage)	HA group	Egg or Cell-origin	Ecto/GH HA1	Source	GISAID/GenBank Accession No.
12-plex	H1 HA head	H1.WI/2019 GH	A/Wisconsin/588/2019	A(H1N1)pdm09	1	Cell	GH HA1	CDC	EPI1661758
12-plex		H1.Syd/2021 GH	A/Sydney/5/2021	A(H1N1)pdm09	1	Cell	GH HA1	CDC	EPI1957293
12-plex	H3 HA head	H3.Cam/2020 GH	A/Cambodia/e0826360/2020	A(H3N2)	2	Cell	GH HA1	CDC	EPI1837753
12-plex		H3.Dar/2021 GH	A/Darwin/6/2021	A(H3N2)	2	Cell	GH HA1	CDC	EPI1857216
12-plex	H5 HA head	H5.AW/2021 GH	A/American Wigeon/South Carolina/22-000345-001/2021	A(H5N1)	1	Cell	GH HA1	CDC	EPI1985910
12-plex	Group 1 HA stalk	Group 1 HA stalk	A/Michigan/45/2015	A(H1N1)pdm09	1	Cell	HA Stalk	CDC	EPI662594
12-plex	Group 2 HA stalk	Group 2 HA stalk	A/Singapore/INFIMH-16-0019/2016	A(H3N2)	2	Cell	HA Stalk	CDC	EPI780183
12-plex	B HA head	BVic WA/2019 GH	B/Washington/02/2019	B/Victoria lineage	N/A	Clinical	GH HA1	CDC	EPI1368874
12-plex		BVic Aus/2021 GH	B/Austria/1359417/2021	B/Victoria lineage	N/A	Cell	GH HA1	CDC	EPI1845793
12-plex		BYam Phu/2013 GH	B/Phuket/3073/2013	B/Yamagata lineage	N/A	Cell	GH HA1	CDC	EPI529345
12-plex	NP	A-NP	A/Brisbane/10/2007	A(H3N2)	N/A	Egg	NP	IRR	EPI176962
12-plex	Protein A/G	Protein A/G	N/A	N/A	N/A	N/A	N/A	ThermoFisher	N/A
28-plex	H1 HA head	H1.ID/2018 GH	A/Idaho/07/2018	A(H1N1)pdm09	1	Cell	GH HA1	CDC	EPI1206974
28-plex		H1. WI/2019 GH	A/Wisconsin/588/2019	A(H1N1)pdm09	1	Cell	GH HA1	CDC	EPI1661758
28-plex		H1.Syd/2021 GH	A/Sydney/5/2021	A(H1N1)pdm09	1	Cell	GH HA1	CDC	EPI1957293
28-plex		H1.WI/2022 GH	A/Wisconsin/67/2022	A(H1N1)pdm09	1	Cell	GH HA1	CDC	EPI2224788
28-plex	H3 HA head	H3.Cam/2020 GH	A/Cambodia/e0826360/2020	A(H3N2)	2	Cell	GH HA1	CDC	EPI1837753
28-plex		H3.Dar/2021 GH	A/Darwin/6/2021	A(H3N2)	2	Cell	GH HA1	CDC	EPI1857216
28-plex		H3.MA/2022 GH	A/Massachusetts/18/2022	A(H3N2)	2	Cell	GH HA1	CDC	EPI2096148
28-plex	H5 HA ectodomain	H5.Indo/2005 Ec	A/Indonesia/05/2005 HA (H5N1)	A(H5N1)	1	Egg	Ecto	CDC	EPI376537
28-plex		H5.TX/2024 Ec	A/Texas/37/2024 (H5N1)	A(H5N1)	1	Egg	Ecto	CDC	EPI3171488
28-plex	H5 HA head	H5.Indo/2005 GH	A/Indonesia/05/2005 HA (H5N1)	A(H5N1)	1	Egg	GH HA1	CDC	EPI376537
28-plex		H5.AW/2021 GH	A/American wigeon/South Carolina/22-000345-001/2021	A(H5N1)	1	Egg	GH HA1	CDC	EPI1985910
28-plex		H5.TX/2024 GH	A/Texas/37/2024 (H5N1)	A(H5N1)	1	Egg	GH HA1	CDC	EPI3171488
28-plex	B HA head	BVic WA/2019 GH	B/Washington/02/2019	B/Victoria lineage	N/A	Clinical	GH HA1	CDC	EPI1368874
28-plex		BVic Aus/2021 GH	B/Austria/1359417/2021	B/Victoria lineage	N/A	Cell	GH HA1	CDC	EPI1845793
28-plex		BYam Phu/2013 GH	B/Phuket/3073/2013	B/Yamagata lineage	N/A	Cell	GH HA1	CDC	EPI529345
28-plex	Seasonal N1	N1 NA.BR/2018	A/Brisbane/02/2018	A(H1N1)pdm09	N/A	Cell	N1 NA	Sino Biological US Inc	EPI1799927
28-plex		N1 NA.WI/2019	A/Wisconsin/588/2019	A(H1N1)pdm09	N/A	Cell	N1 NA	Sino Biological US Inc	EPI1661757
28-plex		N1 NA.Syd/2021	A/Sydney/5/2021	A(H1N1)pdm09	N/A	Cell	N1 NA	Sino Biological US Inc	EPI2413554
28-plex		N1 NA.WI/2022	A/Wisconsin/67/2022	A(H1N1)pdm09	N/A	Cell	N1 NA	Sino Biological US Inc	EPI2224787
28-plex	Seasonal N2	N2 NA.Cam/2020	A/Cambodia/e0826360/2020	A(H3N2)	N/A	Cell	N2 NA	Sino Biological US Inc	EPI1837752
28-plex		N2 NA.Dar/2021	A/Darwin/6/2021	A(H3N2)	N/A	Cell	N2 NA	Sino Biological US Inc	EPI1925254
28-plex		N2 NA.MA/2022	A/Massachusetts/18/2022	A(H3N2)	N/A	Cell	N2 NA	CDC	EPI2096147
28-plex	N1 (H5N1)	N1.NA H5.AW/2021	A/American wigeon/South Carolina/22-000345-001/2021	A(H5N1)	N/A	Cell	N1 NA	CDC	EPI1985912
28-plex		N1 NA H5.TX/2024	A/Texas/37/2024 (H5N1)	A(H5N1)	N/A	Egg	N1 NA	CDC	EPI3171486
28-plex	Group 1 HA stalk	Group 1 HA stalk	A/Michigan/45/2015	A(H1N1)pdm09	1	Cell	HA Stalk	CDC	EPI662594
28-plex	Group 2 HA stalk	Group 2 HA stalk	A/Singapore/INFIMH-16-0019/2016	A(H3N2)	2	Cell	HA Stalk	CDC	EPI780183
28-plex	NP	A-NP	A/Brisbane/10/2007	A(H3N2)	N/A	Egg	NP	IRR	EPI176962
28-plex	Protein A/G	Protein A/G	N/A	N/A	N/A	N/A	N/A	ThermoFisher	N/A

GH: HA globular head

Ec: HA ectodomain

Supplementary Table S2: Sensitivity and Specificity of the MIADA assay in detecting binding antibodies to the HA head of 2.3.4.4b A(H5N1) virus A/American wigeon/SC/22-00345-001/2021

MFI Cutoff to 2.3.4.4b AW/2021 H5N1 GH	Sensitivity % (p^a/n^b)	Specificity % (D^c/Y^d)	J-index^e
500	100.0 (42/42)	98.0 (1438/1467)	0.980
750	100.0 (42/42)	99.3 (1457/1467)	0.993
1000	100.0 (42/42)	99.6 (1461/1467)	0.996
1500	97.6 (41/42)	99.9 (1466/1467)	0.975
2000	93.0 (40/42)	99.9 (1466/1467)	0.951

^aSerum samples showed MFIs > cutoff values

^b42 H5 positive vaccination sera (≥40 neutralizing antibody titers against A/American wigeon/SC/22-00345-001/2021 A(H5N1) 2.3.4.4b wild type virus)

^cSerum samples showed MFIs < cutoff values

^dTotal 1467 sera were collected from 489 participants in 3 waves from November 2021 to February 2023

^e: J-index sensitivity+specificity-1

Supplementary Table S3: Percent amino acid homology of HA1 sequences of A(H5N1) and recent seasonal A(H1N1)pdm09 viruses

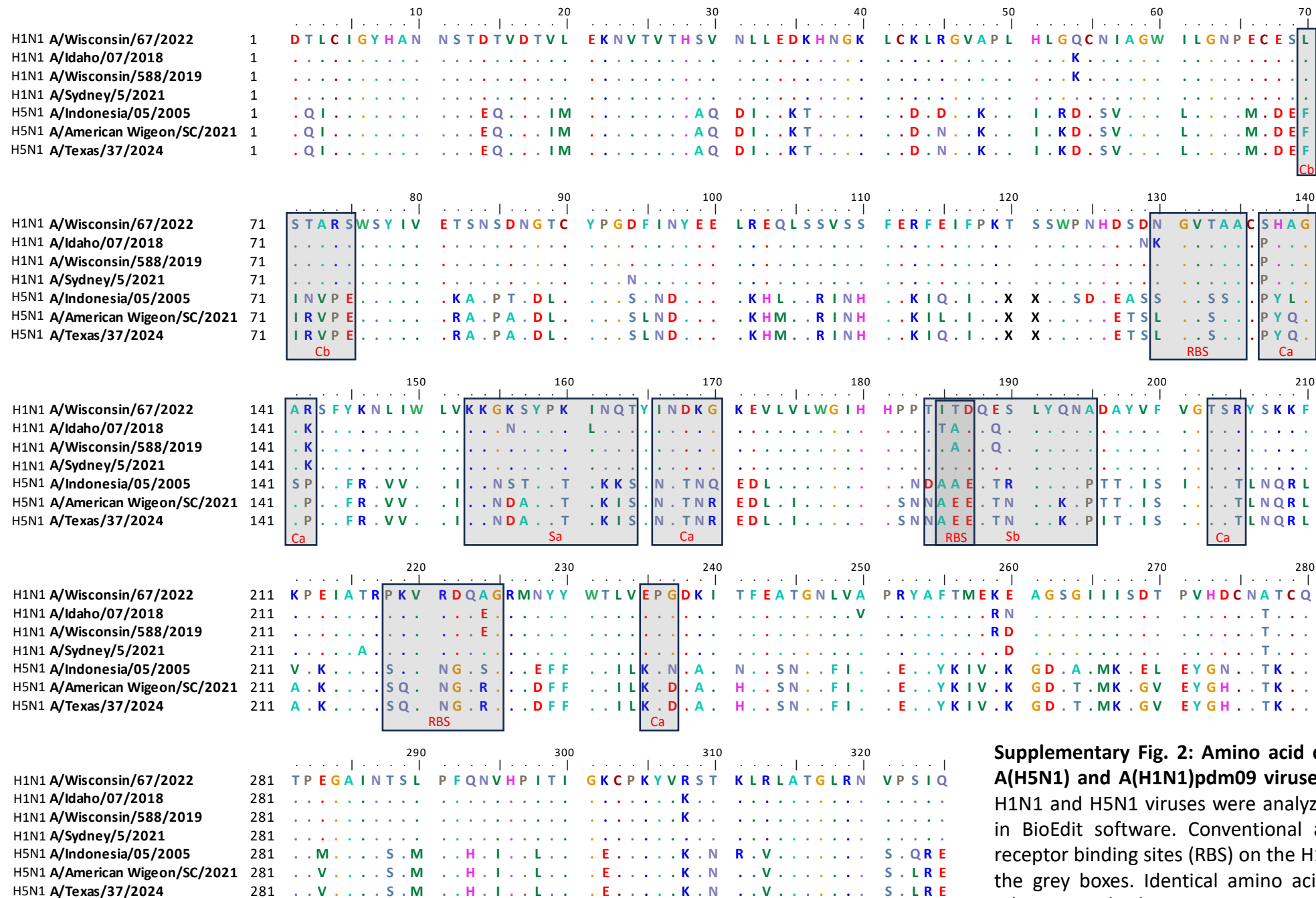
	A/Texas/37/2024 H5N1 (2.3.4.4b)	A/American wigeon/2021 H5N1 (2.3.4.4b)	A/Indonesia/05/2005 H5N1 (2.1)	A/Wisconsin/67/2022 H1N1	A/Sydney/5/2021 H1N1	A/Wisconsin/588/2019 H1N1	A/Idaho/07/2018 H1N1
A/Texas/37/2024 H5N1 (2.3.4.4b)	N.A	99	88	53	53	53	53
A/American wigeon/2021 H5N1 (2.3.4.4b)	99	N.A	88	53	53	53	53
A/Indonesia/05/2005 H5N1 (2.1)	88	88	N.A	52	53	53	53
A/Wisconsin/67/2022 H1N1	53	53	52	N.A	98	97	95
A/Sydney/5/2021 H1N1	53	53	53	98	N.A	98	95
A/Wisconsin/588/2019 H1N1	53	53	53	97	98	N.A	98
A/Idaho/07/2018 H1N1	53	53	53	95	95	98	N.A

N/A: non applicable

Supplementary Table S4: Percent amino acid homology of NA sequences of A(H5N1) and recent seasonal A(H1N1)pdm09 viruses

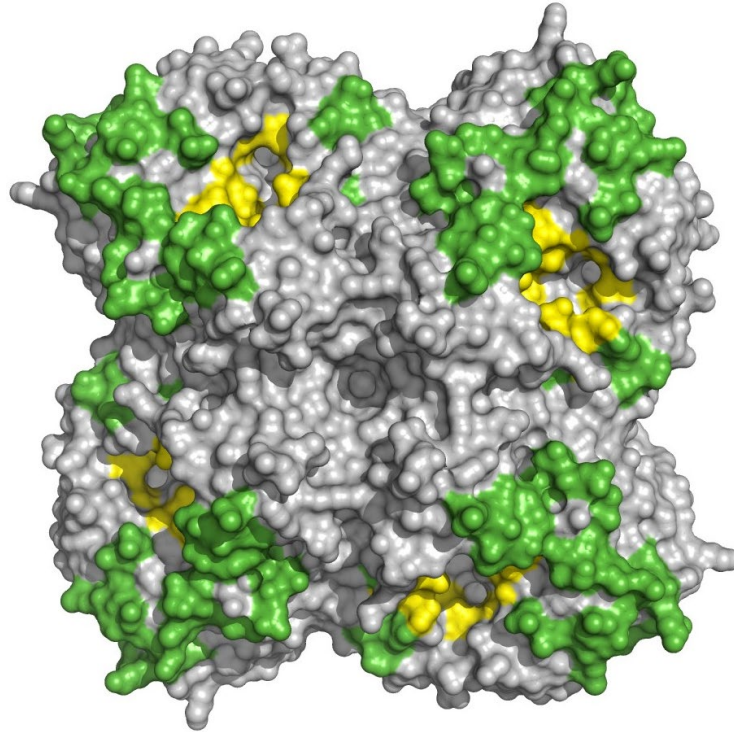
	A/Texas/37/2024 H5N1 (2.3.4.4b)	A/American wigeon/2021 H5N1 (2.3.4.4b)	A/Wisconsin/67/2022 H1N1	A/Sydney/5/2021 H1N1	A/Wisconsin/588/2019 H1N1	A/Brisbane/02/2018 H1N1
A/Texas/37/2024 H5N1 (2.3.4.4b)	N.A	99	86	87	87	88
A/American wigeon/2021 H5N1 (2.3.4.4b)	99	N.A	86	87	87	88
A/Wisconsin/67/2022 H1N1	86	86	N.A	99	99	97
A/Sydney/5/2021 H1N1	87	87	99	N.A	99	98
A/Wisconsin/588/2019 H1N1	87	87	99	99	N.A	98
A/Brisbane/02/2018 H1N1	88	88	97	98	98	N.A

N/A: non applicable

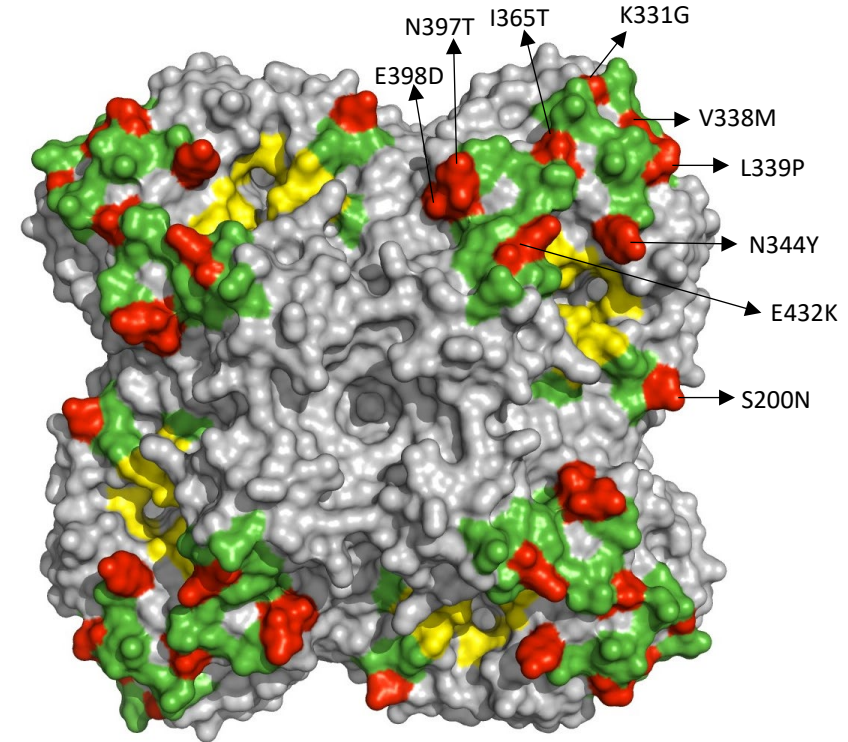


Supplementary Fig. 2: Amino acid differences between the HA head of A(H5N1) and A(H1N1)pdm09 viruses analyzed. HA1 sequences from the H1N1 and H5N1 viruses were analyzed using ClustalW multiple alignment in BioEdit software. Conventional antigenic sites (Ca, Cb, Sa, Sb) and receptor binding sites (RBS) on the H1 HA head domain were highlighted in the grey boxes. Identical amino acids referred to the HA1 sequence of A/Wisconsin/67/2022 H1N1pdm09 virus were presented as dots.

NA of A/Wisconsin/67/2022 A(H1N1)pdm09



NA of A/Texas/37/2024 2.3.4.4b A(H5N1)



Supplementary Fig. 4: Comparison of amino acid residues in the potential NA antigenic sites between A/Wisconsin/67/2022 H1N1 pdm09 and A/Texas/37/2024 H5N1 viruses. The NA tetramers were modeled with PyMOL version 2.1.1 from A/Michigan/45/2015 H1N1 pdm09 (PDB: 7S0I) for A/Wisconsin/67/2022 H1N1 pdm09 NA and from A/American green-winged teal/Washington/195750/2014 H5N1 (PDB: 5HUG) for A/Texas/37/2024 H5N1 NA respectively. Putative NA antigenic sites were shown in green, of which 10 amino acid differences (at site 200, 331, 336, 338, 339, 344, 365, 397, 398, 432) between the two NA sequences were highlighted in red on the modeling of A/Texas/37/2024 H5N1 virus. G336S change was underneath the 338 and 339 sites and was not visible from the top view of this tetramer. The amino acid residues in the enzyme active site were presented in yellow.