

Table S1: Inventory of PA amino acid residues monitored for assessment of baloxavir susceptibility in this study

No.	Type A		Type B		Protein region/ functional role	Rationale	References
	Residue #	Conserved amino acid	Residue #	Conserved amino acid			
Previously reported residues at which certain substitutions were implicated in reduced baloxavir susceptibility							
1	18	E	18	K	N-ter/noncatalytic site	E18G in A(H1N1) RG virus conferred elevated baloxavir EC ₅₀ (10-fold).	Luo et al., 2023
2	23	E	23	E	N-ter/noncatalytic site	Changes at this residue (E23G, K or R) in type A viruses (RG or seasonal isolates) were shown to confer variably elevated baloxavir EC ₅₀ (1.8-19-fold); E23K in B lineage RG viruses did not affect baloxavir EC ₅₀ (0.8-2.6-fold).	Omoto et al., 2018; Gubareva et al., 2019; Ince et al., 2020; Takashita et al., 2020; Abed et al., 2020; Jones et al., 2022a, 2022b
3	28 ^a	L or P	28	P	N-ter/noncatalytic site	L28P in A(H3N2) RG virus conferred mild elevated baloxavir EC ₅₀ (2.6-fold). This substitution in A(H3N2) seasonal isolates did not affect baloxavir EC ₅₀ (0.4-1.0-fold) vs sequence-matched controls.	Omoto et al., 2018; Govorkova et al., 2022
4	34	K	34	M	N-ter/active site	K34R in type A viruses conferred elevated baloxavir EC ₅₀ (3.7-5-fold) vs sequence-matched controls; M34I in B virus did not affect baloxavir EC ₅₀ (0.6-fold).	Govorkova et al., 2022
5	36	A	36	F	N-ter/noncatalytic site	A36V in type A RG viruses conferred elevated baloxavir EC ₅₀ (3.6-6-fold). F36V in B lineage RG virus did not affect baloxavir EC ₅₀ (0.8-fold).	Omoto et al., 2018
6	37	A	37	N	N-ter/active site	A37T in type A viruses (RG or seasonal isolates; also in A(H5N1)) conferred elevated baloxavir EC ₅₀ (5-8-fold).	Omoto et al., 2018; Sato et al., 2021; Nguyen et al., 2023
7	38	I	38	I	N-ter/active site	I38T is recognized as primary molecular marker conferring resistance to baloxavir in both type A and B viruses; other changes at this residue (I38F, L, M, N, or S) in type A viruses (RG or seasonal isolates; also in A(H5N1)) and (I38F or M) in B lineage RG viruses conferred variably elevated baloxavir EC ₅₀ (2.2-112-fold) and (1.7-8-fold), respectively.	Omoto et al., 2018; Gubareva et al., 2019; Jones et al., 2020; Imai et al., 2020; Hashimoto et al., 2021; Govorkova et al., 2022; Nguyen et al., 2023; Taniguchi et al., 2024
8	119	E	120	E	N-ter/metal binding	E119D in type A RG viruses conferred elevated baloxavir EC ₅₀ (5-6-fold). E120D in B lineage RG virus did not affect baloxavir EC ₅₀ (2.0-fold).	Omoto et al., 2018
9	198	E	199	G	N-ter or linker/noncatalytic site	E198K in type A RG viruses conferred variably elevated baloxavir EC ₅₀ (1.8-6-fold). G199R in B lineage RG virus did not affect baloxavir EC ₅₀ (2.0-fold).	Takizawa & Momose 2022; Hickerson et al., 2022
10	199	E	200	I	Linker/noncatalytic site	Changes at this residue (E199D, G or K) in type A viruses (RG or seasonal isolates) conferred variably elevated baloxavir EC ₅₀ (0.5-7-fold).	Omoto et al., 2018; Kiso et al., 2020; Hashimoto et al., 2021; Govorkova et al., 2022; Hickerson et al., 2022 Takashita et al., 2023; Takashita et al., 2025
Additional residues implicated in PA inhibitor binding via crystal structure or mutational analysis							
1	20	A	20	T	N-ter/active site	Baloxavir makes van der Waals contact with Ala20 of type A. T20K in influenza B virus was shown to confer elevated EC ₅₀ (7.0-fold).	Omoto et al., 2018; Palmu et al., 2025
2	24	Y	24	F	N-ter/active site	Baloxavir makes van der Waals contact with Tyr24 of type A.	Omoto et al., 2018
3	41	H	41	H	N-ter/metal binding	Side chain of His41 interacts with Mn ⁺² in the active center; H41A in A(H1N1) RG virus was shown to confer elevated EC ₅₀ for L-742,001.	Stevaert et al., 2013; Omoto et al., 2018

4	42	L	42	L	N-ter/noncatalytic site	Leu42 is adjacent to the metal-chelating residue His41; L42T in A(H1N1) RG virus was associated with mild elevated EC ₅₀ for L-742,001.	Stevaert et al., 2013
5	80	E	81	E	N-ter/metal binding	Side chain of Glu80 or Glu81 interacts with Mn ⁺² in the active center.	Omoto et al., 2018
6	81	G	82	G	N-ter/noncatalytic site	Gly81 is adjacent to the metal-chelating residue Glu80; changes at this residue (G81 F, T or V) in A(H1N1) RG viruses were shown to confer elevated EC ₅₀ for L-742,001.	Stevaert et al., 2013
7	84	R	85	R	N-ter/noncatalytic site	Arg85 makes additional hydrophobic contact with baloxavir in type B but not in type A.	Omoto et al., 2018
8	106	L	107	L	N-ter/noncatalytic site	Involve in hydrogen bond network for baloxavir binding; L106R in type A RG viruses was associated with elevated baloxavir EC ₅₀ .	Omoto et al., 2018; Chen et al., 2024
9	107	P	108	A	N-ter/noncatalytic site	Involve in hydrogen bond network for baloxavir binding.	Omoto et al., 2018
10	108	D	109	D	N-ter/metal binding	Side chain of Asp108 or Asp109 interacts with Mn ⁺² in the active center.	Omoto et al., 2018
11	120	I	121	V	N-ter/metal binding	Side chain of Iso120 or Val121 interacts with Mn ⁺² in the endonuclease active center; I120T in A(H1N1) RG virus was shown to confer elevated EC ₅₀ for L-742,001.	Stevaert et al., 2013; Omoto et al., 2018
12	122	V	123	I	N-ter/noncatalytic site	Involve in hydrogen bond network for baloxavir binding; V122T in A(H1N1) RG virus was associated with mild elevated EC ₅₀ for L-742,001.	Stevaert et al., 2013; Omoto et al., 2018
13	130	Y	131	Y	N-ter/noncatalytic site	Involve in hydrogen bond network for baloxavir binding.	Omoto et al., 2018
14	134	K	135	K	N-ter/noncatalytic site	Involve in hydrogen bond network for baloxavir binding.	Omoto et al., 2018

PA, polymerase acidic subunit; N-ter, N-terminal

^aThe consensus amino acid at residue 28 is proline (P) and leucine (L) for A(H1N1)pdm09 and A(H3N2) virus backgrounds, respectively.

Table S2: Summary of seasonal influenza positive specimens for US national surveillance processed at three National Influenza Reference Centers, 2023-2024 season

Month/year	No. specimens												
	Received by NIRCs ^a				A(H1N1)pdm09			A(H3N2)			B/Victoria		
	CA	NY	WI	Total	Received	Sequenced	Propagated	Received	Sequenced	Propagated	Received	Sequenced	Propagated
Oct. 2023	133	170	90	393	230	230	116	61	59	47	102	104	62
Nov. 2023	180	201	180	561	272	269	131	130	131	91	159	160	97
Dec. 2023	249	293	266	808	320	315	164	288	289	199	200	196	115
Jan. 2024	263	306	351	920	362	353	163	313	312	194	245	242	135
Feb. 2024	235	323	298	856	267	263	137	335	329	203	254	251	147
Mar. 2024	225	336	272	833	238	237	127	339	332	217	256	252	142
Apr. 2024	133	183	159	475	136	133	83	179	176	128	160	159	98
May 2024	114	64	74	252	70	69	47	102	101	77	80	77	54
Jun. 2024	124	35	68	227	71	66	50	117	113	84	39	34	24
Jul. 2024	72	63	47	182	73	66	52	101	93	70	8	7	6
Aug. 2024	59	66	53	178	76	71	50	96	89	74	6	6	6
Sep. 2024	44	57	56	157	90	88	62	58	56	46	9	8	7
Total	1831	2097	1914	5842	2205	2160	1182	2119	2080	1430	1518	1496	893

^aThe location of three state Public Health Laboratories (PHLs) designated as National Influenza Reference Center (NIRCs) are shown with two-letter US state postal abbreviations (CA, California; NY, New York; WI, Wisconsin). The names of three NIRCs are: California Department of Public Health Viral and Rickettsial Disease Laboratory, New York State Department of Health (Wadsworth Center), and Wisconsin State Laboratory of Hygiene.

Table S3: Summary of seasonal influenza positive specimens from other member countries of PAHO processed at CDC, 2023-2024 season

Other PAHO countries	No. specimens received	No. specimens								
		A(H1N1)pdm09			A(H3N2)			B/Victoria		
		Received	Sequenced	Propagated	Received	Sequenced	Propagated	Received	Sequenced	Propagated
Argentina	69	2	2	1	36	33	18	31	30	7
Barbados	17	10	6	6	5	5	1	2	-	-
Bolivia	16	-	-	-	6	6	6	10	10	10
Brazil	180	78	71	28	72	70	26	30	24	3
Canada	93	44	43	44	25	25	24	24	24	24
Chile	87	25	21	9	22	20	15	40	38	32
Colombia	62	26	24	13	19	14	14	17	16	9
Costa Rica	53	24	22	15	22	21	19	7	6	4
Ecuador	32	25	12	-	-	-	-	7	2	-
El Salvador	36	11	11	1	10	10	3	15	15	14
French Guiana	44	21	21	21	23	23	17	-	-	-
Guatemala	43	13	13	10	13	13	8	17	17	10
Guyana	80	39	36	27	38	37	25	5	4	4
Haiti	71	29	24	3	40	-	-	2	1	-
Honduras	26	7	5	-	17	14	7	2	1	-
Jamaica	73	60	60	9	13	12	6	-	-	-
Martinique	8	4	4	4	4	4	4	-	-	-
Mexico	69	3	2	2	28	27	26	38	38	37
Nicaragua	40	2	1	1	38	33	33	-	-	-
Panama	36	11	10	7	24	24	20	1	1	1
Paraguay	30	4	4	1	11	11	6	15	15	9
Peru	59	17	14	5	21	20	18	21	19	13
Suriname	10	9	8	8	1	1	1	-	-	-
Uruguay	50	-	-	-	50	48	22	-	-	-
Venezuela	11	-	-	-	1	1	-	10	7	-
Total	1295	464	414	215	539	472	319	294	268	177

PAHO, Pan American Health Organization

Table S4: Summary of influenza A and B viruses potentially displaying resistance or reduced antiviral susceptibility

Surveillance	Virus name	Type/subtype or lineage	Date of collection (MM/DD/Yr)	Patient's location	Patient Age/Sex	IC ₅₀ fold change ^a				NA substitution ^b		NA sequence GISAID acc. No.		NAI exposure
						Oseltamivir	Zanamivir	Peramivir	Laninamivir	Virus isolate	Clinical specimen	Virus isolate	Clinical specimen	
Viruses with NA-H275Y														
US National	A/Montana/53/2023	A(H1N1)pdm09	12/5/2023	US	72/F	1167 (HRI)	1	320 (HRI)	2	H275Y	H275Y	EPI3728914	EPI2902453	Yes
US National	A/Maine/12/2024	A(H1N1)pdm09	2/22/2024	US	53/M	1387 (HRI)	1	325 (HRI)	2	H275Y	H275Y	EPI3202494	EPI3202494	No
US National	A/Colorado/65/2024	A(H1N1)pdm09	3/25/2024	US	73/M	1	3	2	1	H275Y/H (Y 21.5%)	H275Y/H (Y 13.8%)	EPI3729241	EPI3266172	Unknown
US National	A/Connecticut/39/2024	A(H1N1)pdm09	4/21/2024	US	59/M	-	-	-	-	VNR	H275Y	-	EPI3341674	Unknown
US National	A/Washington/208/2024	A(H1N1)pdm09	5/28/2024	US	1/F	1815 (HRI)	1	445 (HRI)	2	H275Y	H275Y	EPI3729249	EPI3432506	No
US National	A/Oregon/56/2024	A(H1N1)pdm09	7/22/2024	US	69/F	1225 (HRI)	1	285 (HRI)	2	H275Y	H275Y	EPI3728922	EPI3552929	No
US Supplemental (NY NIRC)	A/New York/196/2024	A(H1N1)pdm09	1/5/2024	US	Unknown	-	-	-	-	-	H275Y/H (Y 10.2%)	-	EPI3729955	Yes
US Supplemental (NY NIRC)	A/New York/197/2024	A(H1N1)pdm09	2/5/2024	US	37/F	-	-	-	-	-	H275Y	-	EPI3729948	Yes
US Supplemental (NY NIRC)	A/New York/198/2024	A(H1N1)pdm09	2/28/2024	US	Unknown	-	-	-	-	-	H275Y	-	EPI3729962	Unknown
US Supplemental (NY NIRC)	A/New York/199/2024	A(H1N1)pdm09	3/19/2024	US	78/F	-	-	-	-	-	H275Y	-	EPI3729968	Unknown
US Supplemental (MN ISC)	A/Minnesota/ISC-1114/2023	A(H1N1)pdm09	12/15/2023	US	32/F	-	-	-	-	-	H275Y	-	EPI3121775	Unknown
US Supplemental (MN ISC)	A/Minnesota/ISC-1118/2023	A(H1N1)pdm09	12/16/2023	US	67/F	-	-	-	-	-	H275Y	-	EPI3121728	Unknown
US Supplemental (FL ISC)	A/Florida/ISC-1088/2024	A(H1N1)pdm09	1/7/2024	US	19/M	-	-	-	-	-	H275Y	-	EPI3198782	Unknown
Other PAHO countries	A/Haiti/20618/2023	A(H1N1)pdm09	9/12/2023 ^c	Haiti	4/F	-	-	-	-	VNR	H275Y	-	EPI3517679	Unknown
Other PAHO countries	A/Haiti/70243/2023	A(H1N1)pdm09	10/25/2023	Haiti	5/F	1498 (HRI)	1	420 (HRI)	2	H275Y	H275Y	EPI3728874	EPI3517655	Unknown
Other PAHO countries	A/Haiti/70290/2023	A(H1N1)pdm09	10/26/2023	Haiti	66/F	-	-	-	-	VNR	H275Y	-	EPI3517827	Unknown
Other PAHO countries	A/Haiti/90291/2023	A(H1N1)pdm09	11/3/2023	Haiti	19/M	1486 (HRI)	1	330 (HRI)	3	H275Y	H275Y	EPI3728882	EPI3517639	Unknown
Viruses with other NA substitutions														
US National	A/Connecticut/11/2023	A(H1N1)pdm09	10/14/2023	US	2/F	13 (RI)	3	4	3	I223V, S247N	I223V, S247N	EPI2814076	EPI2813432	Unknown
US National	B/Ohio/128/2024	B/Victoria	4/24/2024	US	45/M	3	9 (RI)	6 (RI)	4	D197N	D197N	EPI3342938	EPI3342188	Unknown
Other PAHO countries	B/Saskatchewan/RV01454/2023	B/Victoria	11/18/2023	Canada	Unknown	5 (RI)	10 (RI)	9 (RI)	4	D197N	Unknown	EPI2928878	-	Unknown
US National	B/California/21/2024	B/Victoria	5/3/2024	US	20/F	2	1	223 (HRI)	1	H273Y	H273Y	Pending	EPI3390752	Unknown

US National	B/Texas/17/2024	B/Victoria	2/22/2024	US	9/M	1	1	14 ^d (RI)	1	A245G	A245G	EPI3206052	EPI3427028	Unknown
US National	B/Texas/18/2024	B/Victoria	2/22/2024	US	11/F	1	1	14 ^d (RI)	1	A245G	A245G	EPI3427021	EPI3206100	Unknown
US Supplemental (TX ISC)	B/Texas/ISC-1014/2024	B/Victoria	2/22/2024	US	6/M	-	-	-	-	-	A245G	-	EPI3296871	Unknown

Viruses with PA substitutions

Surveillance	Virus name	Type/subtype	Date of collection	Patient's location	Patient Age/Sex	Baloxavir EC ₅₀ fold change ^e	PA substitution		PA sequence GISAID acc. No.		Baloxavir exposure
							Virus isolate	Clinical specimen	Virus isolate	Clinical specimen	
US National	A/Hawaii/74/2023	A(H3N2)	12/1/2023	US	62/M	102 (reduced susceptibility)	I38T	I38T	EPI3426994	EPI2877242	No ^f
US National	A/California/55/2024	A(H1N1)pdm09	4/9/2024	US	54/F	9-11 ^g (reduced susceptibility)	V122A	V122A	EPI3519981	EPI3340993	Unknown
US National	A/New Mexico/21/2024	A(H1N1)pdm09	3/7/2024	US	71/M	4 ^h (reduced susceptibility)	Y24C	Y24C	EPI3519528	EPI3266217	Unknown

EC₅₀, 50% effective concentration; GISAID, Global Initiative on Sharing All Influenza Data; HRI, highly reduced inhibition; IC₅₀, 50% inhibitory concentration; NA, neuraminidase; NAI, NA inhibitor; nt, not tested; PA, polymerase acidic subunit; PAHO, Pan American Health Organization; RI, reduced inhibition; VNR, virus not recovered; “-”, not applicable

^aFold changes in IC₅₀s were calculated versus subtype- or lineage-specific median IC₅₀s generated by testing representative viruses that circulated during 2023-2024 season (see Table 2). Fold changes were interpreted according to arbitrary criteria implemented by surveillance laboratories. For type A: <10-fold, normal inhibition; 10-100-fold, reduced inhibition; >100-fold, highly reduced inhibition; for type B: <5-fold, normal inhibition; 5-50-fold, reduced inhibition; >50-fold, highly reduced inhibition.

^bSubstitution is shown using type- or subtype-specific NA amino acid numbering system.

^cThe collection date of this virus falls outside of the reporting period of 2023-2024 season (i.e., October 1, 2023 to September 30, 2024).

^d14-fold compared to both, NA sequence-matched control virus or the lineage-specific median.

^eFold changes in EC₅₀s were calculated versus subtype- or lineage-specific median EC₅₀s generated by testing representative viruses that circulated during 2023-2024 season (see Figure 3). Fold change was interpreted according to provisional criteria, where >3-fold increase in EC₅₀ of test virus versus reference EC₅₀ is defined as reduced susceptibility to baloxavir.

^fRecent travel from Japan.

^g9- and 11-fold compared to PA sequence-matched control virus and the subtype-specific median, respectively.

^h4-fold compared to both, PA sequence-matched control virus or the subtype-specific median.

Table S5: Demographic data for two clusters of NA mutants

Cluster of A(H1N1)pdm09 viruses with NA-H275Y from Jérémie, Haiti						
Virus name	Collection date (MM/DD/Year)	Site of specimen collection	Patient (Age/Sex)	Subtype/lineage- specific Ct value	NAI exposure	NA sequence GISAID EpiFlu™ ID
A/Haiti/20618/2023	9/12/2023 ^a	Hôpital Saint Antoine de Jérémie	4/F	30.61	Unknown	EPI3517679
A/Haiti/70243/2023	10/25/2023	Hôpital Saint Antoine de Jérémie	5/F	24.33	Unknown	EPI3517655
A/Haiti/70290/2023	10/26/2023	Klinik Pèp Bondye HHF	66/F	25.81	Unknown	EPI3517827
A/Haiti/90291/2023	11/3/2023	Hôpital Saint Antoine de Jérémie	19/M	25.30	Unknown	EPI3517639
Cluster of B/Victoria viruses with NA-A245G from Texas, US						
B/Texas/17/2024	2/22/2024	Fort Worth, Tarrant County	9/M	14.4	Unknown	EPI3206052
B/Texas/18/2024	2/22/2024	Fort Worth, Tarrant County	11/F	17.0	Unknown	EPI3206100
B/Texas/ISC-1014/2024 ^b	2/22/2024	Fort Worth, Tarrant County	6/M	nt	Unknown	EPI3296871

GISAID, Global Initiative on Sharing All Influenza Data; NA, neuraminidase; NAI, NA inhibitor; nt, not tested

^aThe collection date of this virus falls outside of the reporting period of 2023-2024 season (i.e., October 1, 2023 to September 30, 2024).

^bVirus was identified from NGS data generated by Texas Influenza Sequencing Center.

Table S6: Baloxavir susceptibility assessment of influenza viruses based on sequence analysis supplemented by IRINA

Type/subtype or lineage	PA sequence analysis				Testing in IRINA	
	PA substitution ^a	US State (No. in each)	Other PAHO countries (No. in each)	No. flagged viruses	No. isolates tested	Baloxavir EC ₅₀ nM (fold) ^b
Previously reported substitutions						
A(H3N2)	I38T	HI (1)	-	1	1	59.38 (102.4)
B/Victoria	M34I	GA (1), KY (1)	Mexico (1)	3	1	1.21 (0.3)
Additional substitutions						
A(H1N1)pdm09	A20T	AR (1), AZ (2), CA (2), CO (2), DC (5), DE (2), FL (1), HI (1), KY (1), LA (1), ME (2), MI (2), MN (1), NE (1), NJ (2), NV (2), NY (3), OH (3), OR (1), PA (6), TX (1), VT (2), WI (1), WV (3)	Brazil (2), Costa Rica (1), French Guiana (1), Jamaica (1)	53	9	0.79 ± 0.19 (1.0)
	Y24C	NM (1)	-	1	1	3.20 (4.0) ^c
	Y24F	-	Guatemala (1)	1	1	1.56 (1.9)
	L106I	MD (1)	-	1	1	0.58 (0.7)
	I120L	ME (1)	-	1	1	0.88 (1.1)
	I120V	NY (1)	-	1	1	0.45 (0.6)
	V122A	CA (1)	-	1	1	8.65 (10.8) ^d
A(H3N2)	V122I	DE (1), ID (1)	-	2	1	0.27 (0.3)
	A20T	DC (1), IA (1), MA (1), MI (1), NV (1), NY (2)	Costa Rica (3), El Salvador (5), Guyana (8), Nicaragua (6), Uruguay (4),	33	2	1.05, 0.54 (1.8, 0.9)
	A20V	DC (1), HI (1), MI (1), NV (2), WA (1), WI(1)	Brazil (1)	8	3	0.59 ± 0.13 (1.0)
	L28M	OH (1), WV (6)	-	7	6	0.70 ± 0.19 (1.2)
	R84K	IN (2), NE (1), OH (1)	-	4	4	0.61 ± 0.04 (1.1)
	I120V	CA (1), NH (1)	Brazil (2)	4	1	0.75 (1.3)
B/Vic	V122L	FL (1)	-	1	1	0.43 (0.7)
	M34V	CO (1)	-	1	1	1.68 (0.4)
	V121I	CO (1), MI (1)	El Salvador (1)	3	3	2.03 ± 0.57 (0.5)
	I123V	DC (2), IA (1), MT(1)	-	4	2	4.86, 4.94 (1.2, 1.2)
	K134R	RI (1)	Costa Rica (1)	2	1	3.09 (0.8)

EC₅₀, 50% effective concentration; IRINA, Influenza Replication Inhibition Neuraminidase-based Assay; PA, Polymerase acidic subunit

^aPA CEN domain sequences of 6567 influenza viruses (2421 A(H1N1)pdm09, 2463 A(H3N2), and 1683 B/Victoria; 5531 from US and 1036 from other countries) were screened to detect previously reported amino acid substitutions that may have an effect on baloxavir susceptibility. In addition, sequences were also screened for either new substitutions at previously reported residues or substitutions at 14 additional residues implicated in baloxavir binding (Table S1). US states are listed using two-letter US postal abbreviations.

^bBaloxavir susceptibility of available isolates of sequence-flagged viruses was assessed using a cell culture-based IRINA. Viruses were tested in at least 2 independent tests. Mean $\pm$ standard deviation of EC₅₀s is shown when three or more viruses with indicated substitution were tested. Otherwise, mean EC₅₀ value is shown. Fold changes in EC₅₀s were calculated versus subtype- or lineage-specific median EC₅₀s generated by testing representative viruses circulated during 2023-2024 season (see Figure 3). Testing outcome was interpreted based on provisional cut-off used previously by surveillance laboratories, according to which >3-fold increase in EC₅₀ of a test virus versus reference EC₅₀ is defined as reduced susceptibility to baloxavir. For amino acid substitutions that conferred reduced baloxavir susceptibility, their respective folds are shown in bold.

^c4-fold increase over EC₅₀ of PA sequence-matched control virus.

^d9-fold increase over EC₅₀ of PA sequence-matched control virus.

Table S7: Inventory of PA amino acid substitutions reported not to have an effect on baloxavir susceptibility (tertiary)

Location in PA ^a	Type A	Type B
CEN domain Type A: 1-198 residues Type B: 1-197 residues	A20 S, T, V	R7K
	Y24F	E23K
	L28 ^f M, P, V	S25G
	K34E	M34 I, V
	F35L	F36 A, V
	A37S	I38V
	I38V	T60V
	V62I	T62K
	V63I	D112N
	P68L	E120D
	L71M	V121I
	R84K	I123V
	V90A	K134R
	T98N	-
	G99E	-
	L106I	-
	I120 L, V	-
	V122 I, L	-
	N136D	-
	D160G	-
	T162I	-
	E181D	-
	A183V	-
	G186D	-
	R192H	-
	E198D	-
Non-CEN domain	Tested using rgA/WSN/33 (H1N1) or rgA/California/04/2009 (H1N1)pdm09	Tested using rgB/Maryland/1/59 or rgB/Victoria/504/2000
	N228I	G199R
	N239Y	D201 E, G
	P267S	K298R
	K328E	T304A
	T363I	E333 G, K
	E397G	Y361H
	A476S	S415 G, N
	V545T	G548R

	E677D	V645A
	-	M682L
	Tested using rgA/Victoria/3/75 (H3N2) or rgA/Hong Kong/218849/2006 (H3N2)	-
	E199K	-
	I201T	-
	R212C	-
	S224F	-
	A231V	-
	C241F	-
	P271S	-
	G299R	-
	G316R	-
	Y321H	-
	R356K	-
	T357A	-
	R385K	-
	S395N	-
	E397K	-
	S405C	-
	N412D	-
	I421T	-
	V432I	-
	L482I	-
	K492R	-
	E493G	-
	V517A	-
	I545M	-
	M561I	-
	M595I	-
	V602I	-
	A618S	-
	E623 G, K	-
	E630K	-
	S632P	-
	L649M	-
	V668I	-
	G684R	-

CEN, cap-dependent endonuclease; PA, polymerase acidic subunit

^aPA CEN domain for type A and type B viruses spans from amino acid residues 1-198 and 1-197, respectively (Omoto et al., 2018). Most substitutions were from two references (Omoto et al., 2018, Hashimoto et al., 2021), and few were from other references (Hickerson et al., 2022, Chen et al., 2024). Tertiary category CEN amino acid substitutions for type A and B viruses tested in this study are shown in bold.

Table S8: Summary of all sequence-flagged influenza A and B viruses with NA or PA substitutions observed in this study through NGS-based antiviral susceptibility surveillance at CDC, 2023-2024 season

Type/subtype or lineage	NA or PA substitutions ^a	Patient Location	Virus Name	Collection Date (MM/DD/Yr)	Patient Age/Sex	GISAID EpiFlu™ Epi Isolate ID (EPI_ISL_)	Originating Lab
A(H1N1)pdm09	NA-H275Y	Haiti	A/Haiti/20618/2023	9/12/2023 ^b	4/F	19355018	Laboratoire National de Sante Publique
A(H1N1)pdm09	NA-H275Y	Haiti	A/Haiti/70243/2023	10/25/2023	5/F	19355015	Laboratoire National de Sante Publique
A(H1N1)pdm09	NA-H275Y	Haiti	A/Haiti/70290/2023	10/26/2023	66/F	19355037	Laboratoire National de Sante Publique
A(H1N1)pdm09	NA-H275Y	Haiti	A/Haiti/90291/2023	11/3/2023	19/M	19355013	Laboratoire National de Sante Publique
A(H1N1)pdm09	NA-H275Y	United States	A/Montana/53/2023	12/5/2023	72/F	18742880	Montana Public Health Laboratory
A(H1N1)pdm09	NA-H275Y	United States	A/Maine/12/2024	2/22/2024	53/M	19014479	Maine Health and Environmental Testing Laboratory
A(H1N1)pdm09	NA-H275Y/H (Y 13.8%)	United States	A/Colorado/65/2024	3/25/2024	73/M	19109265	Colorado Department of Public Health and Environment, Laboratory Services Division
A(H1N1)pdm09	NA-H275Y	United States	A/Connecticut/39/2024	4/21/2024	59/M	19168614	Public Health Laboratory, Connecticut Department of Public Health
A(H1N1)pdm09	NA-H275Y	United States	A/Washington/208/2024	5/28/2024	1/F	19259570	Washington State Public Health Laboratories
A(H1N1)pdm09	NA-H275Y	United States	A/Oregon/56/2024	7/22/2024	69/F	19407744	Oregon State Public Health Laboratory
A(H1N1)pdm09	NA-I223V+S247N	United States	A/Connecticut/11/2023	10/14/2023	2/F	18586500	Public Health Laboratory, Connecticut Department of Public Health
A(H1N1)pdm09	NA-Q136H	United States	A/Arkansas/44/2024	3/13/2024	29/F	19083538	New York State Department of Health, Wadsworth Center
A(H1N1)pdm09	NA-D199V	Brazil	A/Sao Paulo/357809524-IAL/2024	6/10/2024	10/M	19298956	Laboratório Central de Saúde Pública de São Paulo - Instituto Adolfo Lutz
A(H1N1)pdm09	NA-I223T	United States	A/New York/79/2023	12/27/2023	43/M	18886041	New York City Department of Health
A(H1N1)pdm09	NA-I223T	United States	A/California/30/2024	2/11/2024	80/M	19014492	California Department of Public Health
A(H1N1)pdm09	NA-I223T	Canada	A/Ontario/RV03913/2024	6/23/2024	Unknown	19355158	Public Health Agency of Canada, National Microbiology Laboratory
B/Victoria	NA-D197N	Canada	B/Saskatchewan/RV01454/2023	11/18/2023	Unknown	18786265	Public Health Agency of Canada, National Microbiology Laboratory
B/Victoria	NA-D197N	United States	B/Ohio/128/2024	4/24/2024	45/M	19168682	Ohio Department of Health, Public Health Laboratory
B/Victoria	NA-H273Y	United States	B/California/21/2024	5/3/2024	20/F	19208992	California Department of Public Health
B/Victoria	NA-A245G	United States	B/Texas/17/2024	2/22/2024	9/M	19053666	Texas Department of State Health Services, Laboratory Services
B/Victoria	NA-A245G	United States	B/Texas/18/2024	2/22/2024	11/F	19053672	Texas Department of State Health Services, Laboratory Services
B/Victoria	NA-H273Q	Canada	B/Ontario/RV01491/2023	11/6/2023	Unknown	18786315	Public Health Agency of Canada, National Microbiology Laboratory
Viruses with NA substitutions identified through supplemental in-state surveillance							
A(H1N1)pdm09	NA-H275Y	United States	A/Florida/ISC-1088/2024	1/7/2024	19/M	19051160	Florida Department of Health, Tampa
A(H1N1)pdm09	NA-H275Y	United States	A/Minnesota/ISC-1114/2023	12/15/2023	32/F	18988570	Minnesota Department of Health, Public Health Laboratory
A(H1N1)pdm09	NA-H275Y	United States	A/Minnesota/ISC-1118/2023	12/16/2023	67/F	18988564	Minnesota Department of Health, Public Health Laboratory
A(H1N1)pdm09	NA-H275Y/H (Y 10.2%)	United States	A/New York/196/2024	1/5/2024	Unknown	19625602	New York State Department of Health, Wadsworth Center
A(H1N1)pdm09	NA-H275Y	United States	A/New York/197/2024	2/5/2024	37/F	19625583	New York State Department of Health, Wadsworth Center
A(H1N1)pdm09	NA-H275Y	United States	A/New York/198/2024	2/28/2024	Unknown	19625617	New York State Department of Health, Wadsworth Center
A(H1N1)pdm09	NA-H275Y	United States	A/New York/199/2024	3/19/2024	78/F	19625632	New York State Department of Health, Wadsworth Center
B/Victoria	NA-A245G	United States	B/Texas/ISC-1014/2024	2/22/2024	6/M	19141557	Texas Department of State Health Services, Laboratory Services
Viruses with PA substitutions							

A(H1N1)pdm09	PA-A20T	United States	A/Arizona/02/2024	1/1/2024	10/M	18862756	Arizona Department of Health Services, Public Health Laboratory
A(H1N1)pdm09	PA-A20T	United States	A/Arizona/10/2024	1/22/2024	52/F	18919923	Arizona Department of Health Services, Public Health Laboratory
A(H1N1)pdm09	PA-A20T	United States	A/Arkansas/40/2024	3/4/2024	67/F	19050786	New York State Department of Health, Wadsworth Center
A(H1N1)pdm09	PA-A20T	United States	A/California/25/2024	1/25/2024	56/M	18991664	California Department of Public Health
A(H1N1)pdm09	PA-A20T	United States	A/California/26/2024	1/27/2024	45/M	19014510	California Department of Public Health
A(H1N1)pdm09	PA-A20T	United States	A/Colorado/25/2024	1/29/2024	Unknown	18990980	Colorado Department of Public Health and Environment, Laboratory Services Division
A(H1N1)pdm09	PA-A20T	United States	A/Colorado/88/2024	4/27/2024	2/F	19216957	Colorado Department of Public Health and Environment, Laboratory Services Division
A(H1N1)pdm09	PA-A20T	United States	A/Delaware/44/2024	3/19/2024	10/F	19109237	Delaware Public Health Laboratory
A(H1N1)pdm09	PA-A20T	United States	A/District of Columbia/07/2024	2/8/2024	Unknown	19014476	Dallas County Health and Human Services
A(H1N1)pdm09	PA-A20T	United States	A/District of Columbia/24/2023	11/26/2023	Unknown	18785910	Dallas County Health and Human Services
A(H1N1)pdm09	PA-A20T	United States	A/District of Columbia/25/2023	11/29/2023	Unknown	18785866	Dallas County Health and Human Services
A(H1N1)pdm09	PA-A20T	United States	A/District of Columbia/30/2023	12/10/2023	Unknown	18862594	Dallas County Health and Human Services
A(H1N1)pdm09	PA-A20T	United States	A/District of Columbia/31/2023	12/12/2023	Unknown	18862711	Dallas County Health and Human Services
A(H1N1)pdm09	PA-A20T	United States	A/Florida/10/2024	1/28/2024	20/M	19014428	Florida Department of Health, Tampa
A(H1N1)pdm09	PA-A20T	United States	A/Hawaii/18/2024	2/12/2024	34/F	19034544	State of Hawaii Department of Health
A(H1N1)pdm09	PA-A20T	United States	A/Kentucky/04/2024	1/4/2024	Unknown	18886080	Kentucky Division of Laboratory Services
A(H1N1)pdm09	PA-A20T	United States	A/Louisiana/05/2024	1/26/2024	72/F	18990955	Louisiana Department of Health, Office of Public Health Central Lab
A(H1N1)pdm09	PA-A20T	United States	A/Maine/28/2024	3/15/2024	24/M	19083671	Maine Health and Environmental Testing Laboratory
A(H1N1)pdm09	PA-A20T	United States	A/Michigan/28/2024	2/16/2024	9/M	19034532	Michigan Department of Health and Human Services
A(H1N1)pdm09	PA-A20T	United States	A/Michigan/34/2024	2/23/2024	4/M	19034526	Michigan Department of Health and Human Services
A(H1N1)pdm09	PA-A20T	United States	A/Minnesota/34/2024	3/10/2024	Unknown	19050814	Minnesota Department of Health, Public Health Laboratory
A(H1N1)pdm09	PA-A20T	United States	A/Nebraska/10/2024	2/20/2024	3/M	18991856	Nebraska Public Health Lab
A(H1N1)pdm09	PA-A20T	United States	A/Nevada/134/2024	4/27/2024	82/M	19168633	Southern Nevada Public Health Lab
A(H1N1)pdm09	PA-A20T	United States	A/Nevada/137/2024	4/29/2024	6/F	19168635	Southern Nevada Public Health Lab
A(H1N1)pdm09	PA-A20T	United States	A/New Jersey/07/2024	1/18/2024	66/M	18991712	New Jersey Department of Health, Public Health and Environmental Laboratories
A(H1N1)pdm09	PA-A20T	United States	A/New Jersey/21/2024	2/14/2024	58/F	19014482	New Jersey Department of Health, Public Health and Environmental Laboratories
A(H1N1)pdm09	PA-A20T	United States	A/New York/22/2024	1/22/2024	50/M	18991802	New York City Department of Health
A(H1N1)pdm09	PA-A20T	United States	A/New York/90/2024	4/3/2024	88/F	19168586	New York State Department of Health, Wadsworth Center
A(H1N1)pdm09	PA-A20T	United States	A/New York/94/2024	4/11/2024	65/M	19168572	New York State Department of Health, Wadsworth Center
A(H1N1)pdm09	PA-A20T	United States	A/Ohio/31/2023	11/30/2023	58/M	18742773	Ohio Department of Health, Public Health Laboratory
A(H1N1)pdm09	PA-A20T	United States	A/Ohio/42/2024	2/21/2024	47/F	18991847	Ohio Department of Health, Public Health Laboratory
A(H1N1)pdm09	PA-A20T	United States	A/Ohio/54/2024	3/16/2024	74/M	19083556	Ohio Department of Health, Public Health Laboratory
A(H1N1)pdm09	PA-A20T	United States	A/Oregon/36/2024	3/25/2024	75/M	19109259	Oregon State Public Health Laboratory
A(H1N1)pdm09	PA-A20T	United States	A/Pennsylvania/13/2024	1/29/2024	85/F	19014427	Pennsylvania Department of Health, Bureau of Laboratories
A(H1N1)pdm09	PA-A20T	United States	A/Pennsylvania/14/2024	1/30/2024	3/M	19014426	Pennsylvania Department of Health, Bureau of Laboratories
A(H1N1)pdm09	PA-A20T	United States	A/Pennsylvania/22/2024	2/14/2024	93/F	19052442	Pennsylvania Department of Health, Bureau of Laboratories
A(H1N1)pdm09	PA-A20T	United States	A/Pennsylvania/23/2024	2/19/2024	59/F	19014456	Pennsylvania Department of Health, Bureau of Laboratories
A(H1N1)pdm09	PA-A20T	United States	A/Pennsylvania/160/2024	3/19/2024	1/M	19168570	Pennsylvania Department of Health, Bureau of Laboratories
A(H1N1)pdm09	PA-A20T	United States	A/Texas/34/2023	12/23/2023	3/F	18862729	California Department of Public Health
A(H1N1)pdm09	PA-A20T	United States	A/Vermont/19/2024	2/26/2024	74/M	19052433	Vermont Department of Health Laboratory
A(H1N1)pdm09	PA-A20T	United States	A/Vermont/21/2023	10/26/2023	56/F	18862550	Vermont Department of Health Laboratory
A(H1N1)pdm09	PA-A20T	United States	A/West Virginia/15/2024	1/28/2024	59/M	19014682	Commonwealth Serum Laboratories

A(H1N1)pdm09	PA-A20T	United States	A/West Virginia/26/2024	2/11/2024	8/M	19014440	Commonwealth Serum Laboratories
A(H1N1)pdm09	PA-A20T	United States	A/West Virginia/57/2023	12/17/2023	58/F	18862691	Commonwealth Serum Laboratories
A(H1N1)pdm09	PA-A20T	United States	A/Wisconsin/16/2024	1/26/2024	79/F	18934440	Wisconsin State Laboratory of Hygiene
A(H1N1)pdm09	PA-A20T	Brazil	A/Amapa/2024-022958-IEC/2024	5/17/2024	53/F	19377207	Evandro Chagas Institute
A(H1N1)pdm09	PA-A20T	Brazil	A/Mato Grosso Du Sul/500888137-IAL/2024	5/31/2024	27/F	19298955	Laboratório Central de Saúde Pública de São Paulo - Instituto Adolfo Lutz
A(H1N1)pdm09	PA-A20T	Costa Rica	A/Costa Rica/6215/2024	3/30/2024	4/M	19185133	Laboratorio Nacional de Influenza
A(H1N1)pdm09	PA-A20T	French Guiana	A/Guyane/305/2024	5/16/2024	66/M	19298939	23 Avenue Pasteur
A(H1N1)pdm09	PA-A20T	Jamaica	A/Jamaica/2839/2024	1/29/2024	Unknown	18991818	University of the West Indies
A(H1N1)pdm09	PA-A20T	United States	A/Delaware/48/2024	3/24/2024	53/M	19109241	Delaware Public Health Laboratory
A(H1N1)pdm09	PA-A20T	United States	A/Maine/29/2024	3/15/2024	74/F	19083679	Maine Health and Environmental Testing Laboratory
A(H1N1)pdm09	PA-A20T	United States	A/Pennsylvania/46/2024	3/24/2024	55/M	19109245	Pennsylvania Department of Health, Bureau of Laboratories
A(H1N1)pdm09	PA-Y24C	United States	A/New Mexico/21/2024	3/7/2024	71/M	19109271	New Mexico Department of Health, Scientific Laboratory Division
A(H1N1)pdm09	PA-Y24F	Guatemala	A/Guatemala/292/2024	2/26/2024	23/M	19141420	Laboratorio Nacional de Salud, Ministerio de Salud Pública y Asistencia Social
A(H1N1)pdm09	PA-L106I	United States	A/Maryland/14/2024	3/4/2024	Unknown	19083518	Maryland Public Health Laboratory, Maryland Department of Health and Mental Hygiene
A(H1N1)pdm09	PA-I120L	United States	A/Maine/03/2024	2/2/2024	82/M	18991807	Maine Health and Environmental Testing Laboratory
A(H1N1)pdm09	PA-I120V	United States	A/New York/97/2024	4/8/2024	53/M	19168591	New York City Department of Health
A(H1N1)pdm09	PA-V122A	United States	A/California/55/2024	4/9/2024	54/F	19168527	California Department of Public Health
A(H1N1)pdm09	PA-V122I	United States	A/Delaware/19/2024	1/29/2024	91/F	18990993	Delaware Public Health Laboratory
A(H1N1)pdm09	PA-V122I	United States	A/Idaho/56/2023	12/14/2023	23/F	18785872	Idaho Department of Health and Welfare, Bureau of Laboratories
A(H3N2)	PA-A20T	Costa Rica	A/Costa Rica/0199/2024	8/11/2024	23/F	19538872	Laboratorio Nacional de Influenza
A(H3N2)	PA-A20T	Costa Rica	A/Costa Rica/8947/2024	7/26/2024	0/F	19538867	Laboratorio Nacional de Influenza
A(H3N2)	PA-A20T	Costa Rica	A/Costa Rica/8390/2024	7/19/2024	71/M	19538869	Laboratorio Nacional de Influenza
A(H3N2)	PA-A20T	United States	A/District of Columbia/09/2024	2/12/2024	Unknown	19012842	Dallas County Health and Human Services
A(H3N2)	PA-A20T	United States	A/Iowa/58/2023	11/3/2023	30/M	18586440	State Hygienic Laboratory, University of Iowa
A(H3N2)	PA-A20T	United States	A/Massachusetts/56/2024	6/24/2024	26/F	19355286	Massachusetts Department of Public Health
A(H3N2)	PA-A20T	United States	A/Michigan/107/2024	5/30/2024	42/F	19259280	Michigan Department of Health and Human Services
A(H3N2)	PA-A20T	United States	A/Nevada/124/2024	4/11/2024	28/F	19141281	Southern Nevada Public Health Lab
A(H3N2)	PA-A20T	United States	A/New York/107/2024	4/26/2024	87/M	19168426	New York State Department of Health, Wadsworth Center
A(H3N2)	PA-A20T	United States	A/New York/157/2024	8/28/2024	66/F	19445415	New York State Department of Health, Wadsworth Center
A(H3N2)	PA-A20T	El Salvador	A/El Salvador/2670/2023	10/25/2023	42/M	18989356	Laboratorio Cental Dr. Max Bloch
A(H3N2)	PA-A20T	El Salvador	A/El Salvador/2682/2023	10/30/2023	21/M	18989358	Laboratorio Cental Dr. Max Bloch
A(H3N2)	PA-A20T	El Salvador	A/El Salvador/2748/2023	11/21/2023	52/F	18989357	Laboratorio Cental Dr. Max Bloch
A(H3N2)	PA-A20T	El Salvador	A/El Salvador/2824/2023	12/18/2023	19/F	18989359	Laboratorio Cental Dr. Max Bloch
A(H3N2)	PA-A20T	El Salvador	A/El Salvador/2833/2023	12/19/2023	33/M	18989355	Laboratorio Cental Dr. Max Bloch
A(H3N2)	PA-A20T	Guyana	A/Guyana/1548/2023	10/11/2023	3/M	18784791	National Public Health Reference Laboratory, Georgetown, Guyana
A(H3N2)	PA-A20T	Guyana	A/Guyana/1549/2023	10/11/2023	5/M	18785768	National Public Health Reference Laboratory, Georgetown, Guyana
A(H3N2)	PA-A20T	Guyana	A/Guyana/4831/2023	11/20/2023	68/M	18785344	National Public Health Reference Laboratory, Georgetown, Guyana
A(H3N2)	PA-A20T	Guyana	A/Guyana/5092/2023	11/23/2023	28/F	18784759	National Public Health Reference Laboratory, Georgetown, Guyana
A(H3N2)	PA-A20T	Guyana	A/Guyana/5259/2023	11/27/2023	13/M	18785770	National Public Health Reference Laboratory, Georgetown, Guyana
A(H3N2)	PA-A20T	Guyana	A/Guyana/5525/2023	11/29/2023	54/M	18785772	National Public Health Reference Laboratory, Georgetown, Guyana

A(H3N2)	PA-A20T	Guyana	A/Guyana/5526/2023	11/23/2023	32/F	18785750	National Public Health Reference Laboratory, Georgetown, Guyana
A(H3N2)	PA-A20T	Guyana	A/Guyana/6183/2023	12/7/2023	53/M	18784705	National Public Health Reference Laboratory, Georgetown, Guyana
A(H3N2)	PA-A20T	Nicaragua	A/Nicaragua/3525/2024	5/19/2024	14/F	19284280	National Reference Center
A(H3N2)	PA-A20T	Nicaragua	A/Nicaragua/3555/2024	5/12/2024	33/M	19284265	National Reference Center
A(H3N2)	PA-A20T	Nicaragua	A/Nicaragua/3679/2024	5/8/2024	37/M	19284269	National Reference Center
A(H3N2)	PA-A20T	Nicaragua	A/Nicaragua/4152/2024	5/26/2024	65/F	19298607	National Reference Center
A(H3N2)	PA-A20T	Nicaragua	A/Nicaragua/4160/2024	5/29/2024	38/M	19298608	National Reference Center
A(H3N2)	PA-A20T	Nicaragua	A/Nicaragua/4362/2024	5/29/2024	22/F	19284264	National Reference Center
A(H3N2)	PA-A20T	Uruguay	A/Uruguay/433/2024	5/15/2024	27/F	19208716	Departamento de Laboratorio de Salud Publica
A(H3N2)	PA-A20T	Uruguay	A/Uruguay/477/2024	5/20/2024	93/F	19208751	Departamento de Laboratorio de Salud Publica
A(H3N2)	PA-A20T	Uruguay	A/Uruguay/826/2024	6/4/2024	10/M	19377006	Departamento de Laboratorio de Salud Publica
A(H3N2)	PA-A20T	Uruguay	A/Uruguay/1188/2024	6/21/2024	73/M	19377138	Departamento de Laboratorio de Salud Publica
A(H3N2)	PA-A20V	United States	A/District of Columbia/29/2023	12/10/2023	Unknown	18862450	Dallas County Health and Human Services
A(H3N2)	PA-A20V	United States	A/Hawaii/77/2023	12/6/2023	10/M	18742558	State of Hawaii Department of Health
A(H3N2)	PA-A20V	United States	A/Michigan/72/2023	12/1/2023	72/M	18742504	Michigan Department of Health and Human Services
A(H3N2)	PA-A20V	United States	A/Nevada/59/2023	12/11/2023	17/M	18742587	Southern Nevada Public Health Lab
A(H3N2)	PA-A20V	United States	A/Nevada/61/2023	12/13/2023	89/F	18742561	Southern Nevada Public Health Lab
A(H3N2)	PA-A20V	United States	A/Washington/16/2024	1/25/2024	6/F	19034505	Washington State Public Health Laboratories
A(H3N2)	PA-A20V	United States	A/Wisconsin/79/2023	10/7/2023	63/M	18527241	Wisconsin State Laboratory of Hygiene
A(H3N2)	PA-A20V	Brazil	A/Sao Paulo/357344822-IAL/2023	10/8/2023	68/M	18742537	Laboratório Central de Saúde Pública de São Paulo - Instituto Adolfo Lutz
A(H3N2)	PA-L28M	United States	A/Ohio/24/2023	11/3/2023	16/F	18669475	Ohio Department of Health, Public Health Laboratory
A(H3N2)	PA-L28M	United States	A/West Virginia/32/2023	10/27/2023	14/M	18586461	Commonwealth Serum Laboratories
A(H3N2)	PA-L28M	United States	A/West Virginia/34/2023	11/5/2023	24/M	18862318	Commonwealth Serum Laboratories
A(H3N2)	PA-L28M	United States	A/West Virginia/35/2023	11/6/2023	19/F	18862404	Commonwealth Serum Laboratories
A(H3N2)	PA-L28M	United States	A/West Virginia/44/2023	12/1/2023	80/F	18862358	Commonwealth Serum Laboratories
A(H3N2)	PA-L28M	United States	A/West Virginia/45/2023	12/2/2023	7/M	18862333	Commonwealth Serum Laboratories
A(H3N2)	PA-L28M	United States	A/West Virginia/61/2023	12/18/2023	4/F	18862366	Commonwealth Serum Laboratories
A(H3N2)	PA-I38T	United States	A/Hawaii/74/2023	12/1/2023	62/M	18708963	State of Hawaii Department of Health
A(H3N2)	PA-R84K	United States	A/Indiana/29/2024	3/10/2024	80/F	19050761	Indiana State Department of Health Laboratories
A(H3N2)	PA-R84K	United States	A/Indiana/40/2024	3/28/2024	60/M	19083383	Indiana State Department of Health Laboratories
A(H3N2)	PA-R84K	United States	A/Nebraska/16/2024	3/15/2024	25/F	19050756	Nebraska Public Health Lab
A(H3N2)	PA-R84K	United States	A/Ohio/12/2024	1/27/2024	5/F	18990395	Ohio Department of Health, Public Health Laboratory
A(H3N2)	PA-I120V	United States	A/California/76/2024	5/23/2024	34/M	19259434	California Department of Public Health
A(H3N2)	PA-I120V	United States	A/New Hampshire/02/2024	1/4/2024	0/M	18919784	New Hampshire Public Health Laboratories
A(H3N2)	PA-I120V	Brazil	A/Paraiba/2024-019638-IEC/2024	5/2/2024	28/F	19284221	Evandro Chagas Institute
A(H3N2)	PA-I120V	Brazil	A/Paraiba/2024-019660-IEC/2024	4/23/2024	31/F	19284222	Evandro Chagas Institute
A(H3N2)	PA-V122L	United States	A/Florida/74/2023	12/6/2023	7/M	18862345	Florida Department of Health, Tampa
B/Victoria	PA-M34I	United States	B/Georgia/47/2023	11/8/2023	70/F	18586747	Georgia Public Health Laboratory
B/Victoria	PA-M34I	United States	B/Kentucky/05/2024	1/15/2024	48/F	18991467	Kentucky Division of Laboratory Services

B/Victoria	PA-M34I	Mexico	B/Mexico/3331/2024	4/25/2024	29/M	19284699	Instituto de Diagnóstico y Referencia Epidemiológicos
B/Victoria	PA-M34V	United States	B/Colorado/11/2024	1/27/2024	5/M	18990863	Colorado Department of Public Health and Environment, Laboratory Services Division
B/Victoria	PA-V121I	United States	B/Michigan/32/2023	12/13/2023	13/F	18863013	Michigan Department of Health and Human Services
B/Victoria	PA-I123V	United States	B/District of Columbia/03/2024	2/8/2024	Unknown	19014762	Dallas County Health and Human Services
B/Victoria	PA-I123V	United States	B/District of Columbia/18/2024	5/6/2024	Unknown	19208976	Dallas County Health and Human Services
B/Victoria	PA-I123V	United States	B/Iowa/63/2023	11/19/2023	52/F	18669746	State Hygienic Laboratory, University of Iowa
B/Victoria	PA-I123V	United States	B/Montana/12/2023	11/27/2023	19/F	18786226	Montana Public Health Laboratory
B/Victoria	PA-K134R	United States	B/Rhode Island/19/2024	4/23/2024	19/F	19185275	Rhode Island Department of Health
B/Victoria	PA-V121I	United States	B/Colorado/53/2024	9/13/2024	4/F	19539485	Colorado Department of Public Health and Environment, Laboratory Services Division
B/Victoria	PA-V121I	El Salvador	B/El Salvador/4152/2024	9/17/2024	19/M	19539501	Laboratorio Cental Dr. Max Bloch
B/Victoria	PA-K134R	Costa Rica	B/Costa Rica/0740/2024	8/12/2024	23/M	19495939	Laboratorio Nacional de Influenza

GISAID, Global Initiative on Sharing All Influenza Data; NA, neuraminidase; PA, polymerase acidic subunit

^aSubstitution is shown using type- or subtype-specific NA amino acid numbering system.

^bThe collection date of this virus falls outside of the reporting period of 2023-2024 season (i.e., October 1, 2023 to September 30, 2024).

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