

Table S1. Primers and probes used for RNase P, detection and subtyping of influenza A and influenza B.**RNase P**

Primer	Sequence (5' to 3')
RNase P For	AGA TTT GGA CCT GCG AGC G
RNase P Rev	GAG CGG CTG TCT CCA CAA GT
RNase P Probe	Cy5 - TTC TGA CCT GAA GGC TCT GCG CG - BHQ-1

Influenza A and B detection

Primer	Sequence (5' to 3')
InfA For1 Primer	CAA GAC CAA TCY TGT CAC CTC TGA C
InfA For2 Primer	CAA GAC CAA TYC TGT CAC CTY TGA C
InfA Rev1 Primer	GCA TTY TGG ACA AAV CGT CTA CG
InfA Rev2 Primer	GCA TTT TGG ATA AAG CGT CTA CG
InfA Probe	FAM -TGC AGT CCT CGC TCA CTG GGC ACG - BHQ-1
InfB For Primer	TCC TCA AYT CAC TCT TCG AGC G
InfB Rev Primer	CGG TGC TCT TGA CCA AAT TGG
InfB Probe	JOE - CCA ATT CGA GCA GCT GAA ACT GCG GTG - BHQ-1

Influenza A subtyping

Primer	Sequence (5' to 3')
<i>A(H1N1)pdm09</i>	
H1pdm For Primer	GTG CTA TAA ACA CCA GYC TCC CAT T
H1pdm Rev Primer	AGA YGG GAC ATT CCT CAA TCC TG
H1pdm Probe	FAM - TGG CCA GYC "T" CA ATT TTG TGC TTT TTA CAT A *
<i>A(H3N2)</i>	
H3 For Primer	AAG CAT TCC YAA TGA CAA ACC
H3 Rev Primer	ATT GCR CCR AAT ATG CCT CTA GT
H3 Probe	FAM - CAG GAT CAC A"T"A TGG GSC CTG TCC CAG *

* "T" residue modified with an internal Black Hole Quencher-1 (10th nucleotide) and with a Spacer C3 at the 3'-end

Influenza B subtyping (lineage)

Primer	Sequence (5' to 3')
<i>Victoria lineage</i>	
B/VIC For Primer	GAT CTG GAT GTA GCC TTG GG
B/VIC Rev Primer	TAA CAG GTC TGA CTT CAT GGA G
B/VIC Probe	FAM - TTC CCT GTR CAT TTT G - BHQ-1
<i>Yamagata lineage</i>	
B/YAM For Primer	GAT CTG GAT GTG GCC TTG GG
B/YAM Rev Primer	AGG TCT GAC YTC GTG RAG TA
B/YAM Probe	FAM - CAC ACA CAT TGG CCT - BHQ-1

Table S2. Protocol used for multi-segment reverse transcription-PCR (M-RT-PCR) of influenza A viruses.

Primer	Sequence (5' to 3')
Uni12/Inf-1	GGG GGG AGC AAA AGC AGG
Uni12/Inf-3	GGG GGG AGC GAA AGC AGG
Uni13/Inf-1	CGG GTT ATT AGT AGA AAC AAG G

These primers target the conserved regions of 12 nucleotides at the 3' terminus and 13 nucleotides at the 5' of each of the eight segments of influenza A genome.

In short, each M-RT-PCR reaction was performed using the SuperScript™ IV One-Step RT-PCR System (Invitrogen, Waltham, MA, USA) in a final volume of 25 µL, including 12,5 µL of Platinum SuperFi RT-PCR Master Mix, 0,5 µL of SuperScript IV RT Mix, 0,2 µL of 10 µM Uni12/Inf-1, 0,3 µL of 10 µM Uni12/Inf-3, 1 µL of 10 µM Uni13/Inf-1, 5 µL of RNA template and 6,5 µL of nuclease-free water.

The thermal profile was as follows: 42°C for 60 min, 94°C for 2 min, and then 5 cycles of 94°C for 30 s, 44 °C for 30 s and 68 °C for 3 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 68°C for 3 min, with a final extension at 68°C for 5 min.

Table S3. Patient's info and GISAID accession numbers of eight segments of influenza A(H1N1)pdm09 viruses collected in Sicily during the season 2023-2024

Patient's info		Influenza A(H1N1)pdm09									
Age (years)	Vaccinated	Strain name	GISAID ID	PB2	PB1	PA	HA	NP	NA	MP	NS
53	No	A/Agrigento/105690/2023	EPI_ISL_19327639	EPI3495214	EPI3495215	EPI3495216	EPI3495218	EPI3495217	EPI3495219	EPI3495220	EPI3495221
9	No	A/Agrigento/106188/2023	EPI_ISL_19327662	EPI3495222	---	EPI3495223	EPI3495224	EPI3495225	EPI3495226	EPI3495227	EPI3495228
5	No	A/Agrigento/106189/2023	EPI_ISL_19327706	EPI3495229	---	EPI3495230	EPI3495231	EPI3495232	EPI3495233	EPI3495234	EPI3495235
92	No	A/Agrigento/106419/2023	EPI_ISL_19327752	EPI3495236	EPI3495237	EPI3495238	EPI3495239	EPI3495240	EPI3495241	EPI3495242	EPI3495243
96	Yes	A/Catania/106036/2023	EPI_ISL_19327877	EPI3495244	---	EPI3495245	EPI3495246	EPI3495247	EPI3495248	EPI3495249	EPI3495250
7	No	A/Catania/106129/2023	EPI_ISL_19327878	EPI3495251	---	---	EPI3495252	EPI3495253	EPI3495254	EPI3495255	EPI3495256
4	No	A/Catania/106699/2023	EPI_ISL_19327879	EPI3501476	---	EPI3501477	EPI3501478	EPI3501479	EPI3501480	EPI3501481	EPI3501482
9	Yes	A/Catania/106704/2023	EPI_ISL_19327880	EPI3501483	---	EPI3501484	EPI3501485	EPI3501486	EPI3501487	EPI3501488	EPI3501489
12	No	A/Catania/106779/2023	EPI_ISL_19327881	EPI3501490	EPI3501491	EPI3501492	EPI3501493	EPI3501494	EPI3501495	EPI3501496	EPI3501497
83	No	A/Catania/106783/2023	EPI_ISL_19327882	EPI3501498	EPI3501499	EPI3501500	EPI3501501	EPI3501502	EPI3501503	EPI3501504	EPI3501505
1	No	A/Catania/107539/2024	EPI_ISL_19327883	EPI3501506	EPI3501507	EPI3501508	---	EPI3501509	---	EPI3501510	EPI3501511
71	Yes	A/Catania/107853/2024	EPI_ISL_19327884	EPI3501512	---	EPI3501513	EPI3501514	EPI3501515	EPI3501516	EPI3501517	EPI3501518
64	Yes	A/Enna/105929/2023	EPI_ISL_19327885	EPI3501519	---	EPI3501520	EPI3501521	EPI3501522	EPI3501523	EPI3501524	EPI3501525
53	Yes	A/Enna/106169/2023	EPI_ISL_19327886	---	---	EPI3501526	EPI3501527	EPI3501528	EPI3501529	EPI3501530	EPI3501531
17	No	A/Messina/106230/2023	EPI_ISL_19327888	EPI3501532	---	EPI3501533	EPI3501534	EPI3501535	EPI3501536	EPI3501537	EPI3501538
2 months	No	A/Messina/106520/2023	EPI_ISL_19327889	---	---	---	---	---	---	EPI3501539	---
1	No	A/Messina/107233/2024	EPI_ISL_19327890	EPI3501540	---	---	EPI3501541	EPI3501542	EPI3501543	EPI3501544	EPI3501545
1	No	A/Messina/107873/2024	EPI_ISL_19327892	---	---	---	EPI3501546			EPI3501547	EPI3501548
6	No	A/Palermo/105654/2023	EPI_ISL_19327895	EPI3501572	---	EPI3501574	EPI3501575	EPI3501576	EPI3501577	EPI3501578	EPI3501579
8 months	No	A/Palermo/105656/2023	EPI_ISL_19327896	EPI3501580	---	EPI3501581	EPI3501582	EPI3501583	EPI3501585	EPI3501589	EPI3501591
3	No	A/Palermo/105910/2023	EPI_ISL_19327897	---	---	---	EPI3501595	---	---	EPI3501596	EPI3501597
40	No	A/Palermo/106054/2023	EPI_ISL_19327898	EPI3501599	---	EPI3501600	EPI3501601	EPI3501602	EPI3501603	EPI3501604	EPI3501605
11	No	A/Palermo/106080/2023	EPI_ISL_19327899	EPI3501606	---	EPI3501607	EPI3501609	EPI3501611	EPI3501613	EPI3501615	EPI3501617
19	No	A/Palermo/106152/2023	EPI_ISL_19327900	EPI3501628	---	---	---	---	EPI3501631	EPI3501632	EPI3501633
12	No	A/Palermo/106200/2023	EPI_ISL_19327901	EPI3501634	---	EPI3501635	EPI3501636	EPI3501637	EPI3501638	EPI3501639	EPI3501640
3	No	A/Palermo/106208/2023	EPI_ISL_19327902	EPI3501641	---	EPI3501642	EPI3501643	EPI3501644	EPI3501645	EPI3501646	EPI3501647
10 months	No	A/Palermo/106209/2023	EPI_ISL_19327903	EPI3501648	---	EPI3501649	EPI3501650	EPI3501651	EPI3501652	EPI3501653	EPI3501654
3	No	A/Palermo/106213/2023	EPI_ISL_19327904	EPI3501655	---	EPI3501656	EPI3501658	EPI3501660	EPI3501663	EPI3501665	EPI3501667
2	No	A/Palermo/106286/2023	EPI_ISL_19327905	EPI3501670	---	EPI3501671	EPI3501672	EPI3501673	EPI3501674	EPI3501675	EPI3501676

Patient's info		Influenza A(H1N1)pdm09									
Age (years)	Vaccinated	Strain name	GISAID ID	PB2	PB1	PA	HA	NP	NA	MP	NS
4	No	A/Palermo/106288/2023	EPI_ISL_19327906	EPI3501682	---	EPI3501684	EPI3501686	EPI3501688	EPI3501689	EPI3501690	EPI3501691
7	No	A/Palermo/106289/2023	EPI_ISL_19328003	EPI3501692	---	EPI3501693	EPI3501694	EPI3501695	EPI3501696	EPI3501697	EPI3501698
2	No	A/Palermo/106432/2023	EPI_ISL_19328004	EPI3501701	---	EPI3501702	EPI3501703	EPI3501704	EPI3501705	EPI3501706	EPI3501707
2	No	A/Palermo/106472/2023	EPI_ISL_19328005	EPI3501708	---	EPI3501709	EPI3501710	EPI3501711	EPI3501712	EPI3501713	EPI3501714
8	No	A/Palermo/106495/2023	EPI_ISL_19328006	EPI3501715	---	EPI3501716	EPI3501717	EPI3501718	EPI3501719	EPI3501720	EPI3501721
2	No	A/Palermo/106507/2023	EPI_ISL_19328007	---	---	EPI3501722	EPI3501723	---	---	EPI3501724	EPI3501725
1	No	A/Palermo/106511/2023	EPI_ISL_19328008	---	---	-----	---	---	---	EPI3501726	---
7	No	A/Palermo/106513/2023	EPI_ISL_19328009	EPI3501727	---	EPI3501728	EPI3501729	EPI3501730	EPI3501731	EPI3501732	EPI3501733
9	No	A/Palermo/106514/2023	EPI_ISL_19328010	EPI3501734	---	EPI3501735	EPI3501736	EPI3501737	EPI3501738	EPI3501739	EPI3501740
7 months	No	A/Palermo/106594/2024	EPI_ISL_19328011	EPI3501741	---	EPI3501742	EPI3501743	EPI3501744	EPI3501745	EPI3501746	EPI3501747
3	No	A/Palermo/106598/2024	EPI_ISL_19328028	EPI3501748	---	EPI3501749	EPI3501750	EPI3501751	EPI3501752	EPI3501753	EPI3501754
5	No	A/Palermo/106634/2023	EPI_ISL_19328042	EPI3501755	EPI3501756	EPI3501757	EPI3501758	EPI3501759	EPI3501760	EPI3501761	EPI3501762
5	No	A/Palermo/106637/2023	EPI_ISL_19328054	EPI3501763	---	---	EPI3501764	EPI3501765	EPI3501766	EPI3501767	EPI3501768
6	No	A/Palermo/106644/2023	EPI_ISL_19328063	EPI3501769	---	EPI3501770	EPI3501771	EPI3501772	EPI3501773	EPI3501774	EPI3501775
9	No	A/Palermo/106673/2024	EPI_ISL_19328073	EPI3501776	---	EPI3501777	EPI3501778	EPI3501779	EPI3501780	EPI3501781	EPI3501782
3	No	A/Palermo/106681/2024	EPI_ISL_19328082	---	---	EPI3501783	EPI3501784	EPI3501785	EPI3501786	EPI3501787	EPI3501788
2	No	A/Palermo/106693/2024	EPI_ISL_19328092	EPI3501789	---	EPI3501790	EPI3501791	EPI3501792	EPI3501793	EPI3501794	EPI3501795
1	Yes	A/Palermo/106742/2024	EPI_ISL_19328101	---	---	EPI3501796	EPI3501797	EPI3501798	EPI3501799	EPI3501800	EPI3501801
6 months	No	A/Palermo/106763/2024	EPI_ISL_19328112	EPI3501802	EPI3501803	EPI3501804	EPI3501805	EPI3501806	EPI3501807	EPI3501808	EPI3501809
4	No	A/Palermo/106766/2024	EPI_ISL_19328123	EPI3501810	EPI3501811	EPI3501812	EPI3501813	EPI3501814	EPI3501815	EPI3501816	EPI3501817
79	No	A/Palermo/106942/2024	EPI_ISL_19328133	EPI3501818	EPI3501819	EPI3501820	EPI3501821	EPI3501822	EPI3501823	EPI3501824	EPI3501825
7	Yes	A/Palermo/107123/2024	EPI_ISL_19328149	EPI3501826	EPI3501827	EPI3501828	EPI3501829	EPI3501830	EPI3501831	EPI3501832	EPI3501833
1	No	A/Palermo/107124/2024	EPI_ISL_19328160	EPI3501834	---	EPI3501835	EPI3501836	EPI3501837	EPI3501838	EPI3501839	EPI3501840
9	No	A/Palermo/107126/2024	EPI_ISL_19330419	EPI3501841	---	EPI3501842	EPI3501843	EPI3501844	EPI3501845	EPI3501846	EPI3501847
8	No	A/Palermo/107218/2024	EPI_ISL_19330426	EPI3501848	EPI3501849	EPI3501850	EPI3501851	EPI3501852	EPI3501853	EPI3501854	EPI3501855
7	No	A/Palermo/107282/2024	EPI_ISL_19330441	EPI3501856	---	EPI3501857	EPI3501858	EPI3501859	EPI3501860	EPI3501861	EPI3501862
2	No	A/Palermo/107283/2024	EPI_ISL_19330442	EPI3501863	EPI3501864	EPI3501865	EPI3501866	EPI3501867	EPI3501868	EPI3501869	EPI3501870
2 months	No	A/Palermo/107286/2024	EPI_ISL_19330443	EPI3501871	EPI3501872	EPI3501873	EPI3501874	EPI3501875	EPI3501876	EPI3501877	EPI3501878
3	No	A/Palermo/107287/2024	EPI_ISL_19330452	EPI3501879	EPI3501880	EPI3501881	EPI3501882	EPI3501883	EPI3501884	EPI3501885	EPI3501886
9 months	No	A/Palermo/107332/2024	EPI_ISL_19330463	EPI3501887	EPI3501888	EPI3501889	EPI3501890	EPI3501891	EPI3501892	EPI3501893	EPI3501894

Patient's info		Influenza A(H1N1)pdm09									
Age (years)	Vaccinated	Strain name	GISAID ID	PB2	PB1	PA	HA	NP	NA	MP	NS
11	No	A/Palermo/107405/2024	EPI_ISL_19330473	EPI3501895	---	EPI3501896	EPI3501897	EPI3501898	---	EPI3501899	EPI3501900
1	No	A/Palermo/107541/2024	EPI_ISL_19330483	---	---	---	EPI3501901	---	EPI3501902	EPI3501903	EPI3501904
9	No	A/Palermo/107571/2024	EPI_ISL_19330493	EPI3501905	---	EPI3501906	EPI3501907	EPI3501908	EPI3501909	EPI3501910	EPI3501911
10	No	A/Palermo/107573/2024	EPI_ISL_19330504	EPI3501912	EPI3501913	EPI3501914	EPI3501915	EPI3501916	EPI3501917	EPI3501918	EPI3501919
10 months	No	A/Palermo/107898/2024	EPI_ISL_19330528	EPI3501920	EPI3501921	EPI3501922	EPI3501923	EPI3501924	EPI3501925	EPI3501926	EPI3501927
4	No	A/Palermo/108000/2024	EPI_ISL_19330539	EPI3501928	---	EPI3501929	EPI3501930	EPI3501931	EPI3501932	EPI3501933	EPI3501934
7	No	A/Palermo/108070/2024	EPI_ISL_19330549	EPI3501935	EPI3501936	EPI3501937	EPI3501938	EPI3501939	EPI3501940	EPI3501941	EPI3501942
62	No	A/Siracusa/107428/2024	EPI_ISL_19330560	EPI3501943	---	EPI3501944	EPI3501945	EPI3501946	EPI3501947	EPI3501948	EPI3501949
12	No	A/Siracusa/107876/2024	EPI_ISL_19330571	EPI3501950	EPI3501951	EPI3501952	EPI3501953	EPI3501954	EPI3501955	EPI3501956	EPI3501957
15	No	A/Siracusa/108015/2024	EPI_ISL_19330580	EPI3501958	---	EPI3501959	EPI3501960	EPI3501961	EPI3501962	EPI3501963	EPI3501964
14	No	A/Trapani/105912/2023	EPI_ISL_19330591	EPI3501965	---	EPI3501966	EPI3501967	EPI3501968	EPI3501969	EPI3501970	EPI3501971
6	No	A/Trapani/106245/2023	EPI_ISL_19330602	EPI3501972	---	EPI3501973	EPI3501974	EPI3501975	EPI3501976	EPI3501977	EPI3501978
2	No	A/Trapani/106607/2024	EPI_ISL_19330611	EPI3501979	---	---	EPI3501980	EPI3501981	EPI3501982	EPI3501983	EPI3501984
4	No	A/Trapani/106612/2024	EPI_ISL_19330621	EPI3501985	---	---	EPI3501986	EPI3501987	EPI3501988	EPI3501989	EPI3501990
9 months	No	A/Trapani/106631/2023	EPI_ISL_19330634	EPI3501991	---	EPI3501992	EPI3501993	EPI3501994	EPI3501995	EPI3501996	EPI3501997
1	No	A/Trapani/107169/2024	EPI_ISL_19330643	EPI3501998	---	EPI3501999	EPI3502000	EPI3502001	EPI3502002	EPI3502003	EPI3502004
1	No	A/Trapani/107205/2024	EPI_ISL_19330653	EPI3502005	---	EPI3502006	EPI3502007	EPI3502008	---	EPI3502009	EPI3502010
33	No	A/Trapani/108213/2024	EPI_ISL_19330667	---	---	---	---	---	---	EPI3502011	EPI3502012

Abbreviations: PB2, polymerase basic 2; PB1, polymerase basic 1; PA, polymerase acid; HA, hemagglutinin; NP, nucleocapsid protein; NA, neuraminidase; MP, matrix protein; NS, nonstructural protein

Table S4. Frequency of AA substitutions identified in A(H1N1)pdm09 strains from Sicily (other than HA and NA segments) during the season 2023-2024 (only mutations with frequencies $\geq 10\%$ are reported).

Segment	Amino acid substitution	Frequency (%)
1 - PB2, polymerase basic 2	I81M/T	64.2
	R251K	16.4
	L475M	46.3
	T662A/S	17.9
2 - PB1, polymerase basic 1	K57T	65.0
	N213D	15.0
	K757R	60.0
3 - PA, polymerase acid	V14I	27.7
	M61I	44.6
	L63I	43.1
	K213R	38.5
	R262K	44.6
	K339N	29.2
	V407I	15.4
	L482M	40.0
5 - NP, nucleoprotein	E14D	46.4
	T22A	46.4
	I33V	40.6
	G34S	15.9
	I136L	46.4
	V217I	46.4
	Q453P	91.3
8 - NS, non-structural protein	W102C (NS1)	16.0
	Q109K (NS1)	37.3
	S212P (NS1)	82.7
	G67E/K (NS2/NEP)	84.0
	Q85H (NS2/NEP)	45.3
	I106L (NS2/NEP)	45.3