Antiviral Drug Resistance

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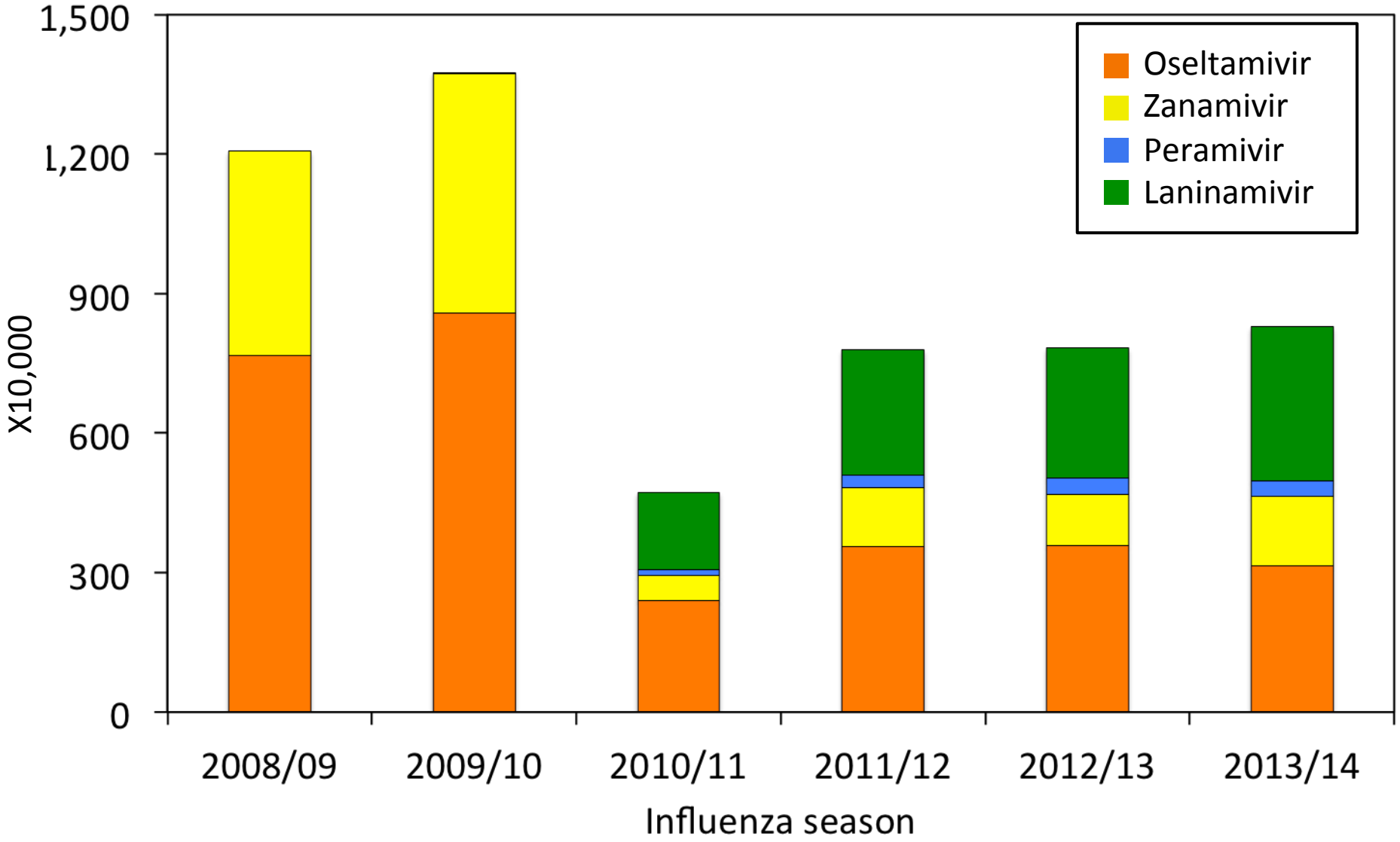
Neuraminidase inhibitor (NAI) Prescriptions in Japan

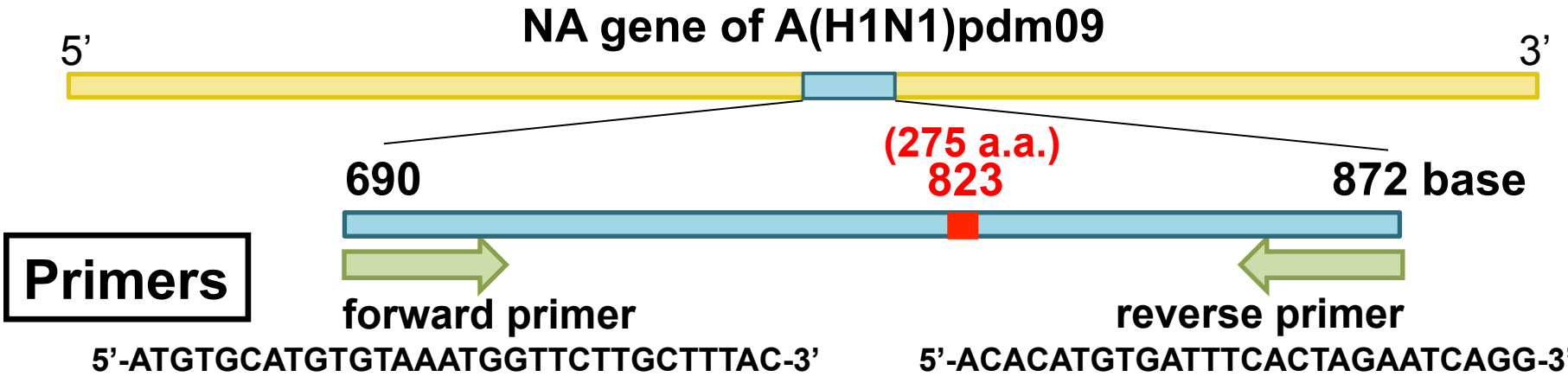


Data from MOHLW, Japan.

Drug resistant mutations in NA

A(H1N1)pdm09	H275Y
A(H3N2)	E119I, E119V, R224K, R292K, N294S,…
B	E117A, E117V, R150K, I221L, R292K, R374K,…

Allele-specific RT-PCR to detect H275Y



Probes

```
  274 275 276 277 278
..TAT TAC TAT GAG GAA..
..  Y   Y   Y   E   E ..


..TAT CAC TAT GAG GAA..
..  Y   H   Y   E   E ..
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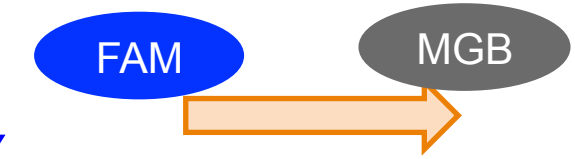
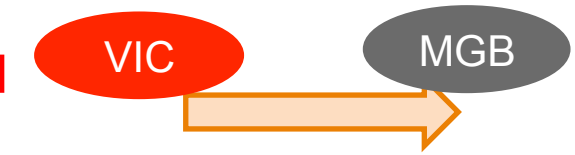
→ 275Y



5’-TACTATGAGGAAT-3’

→ 275H

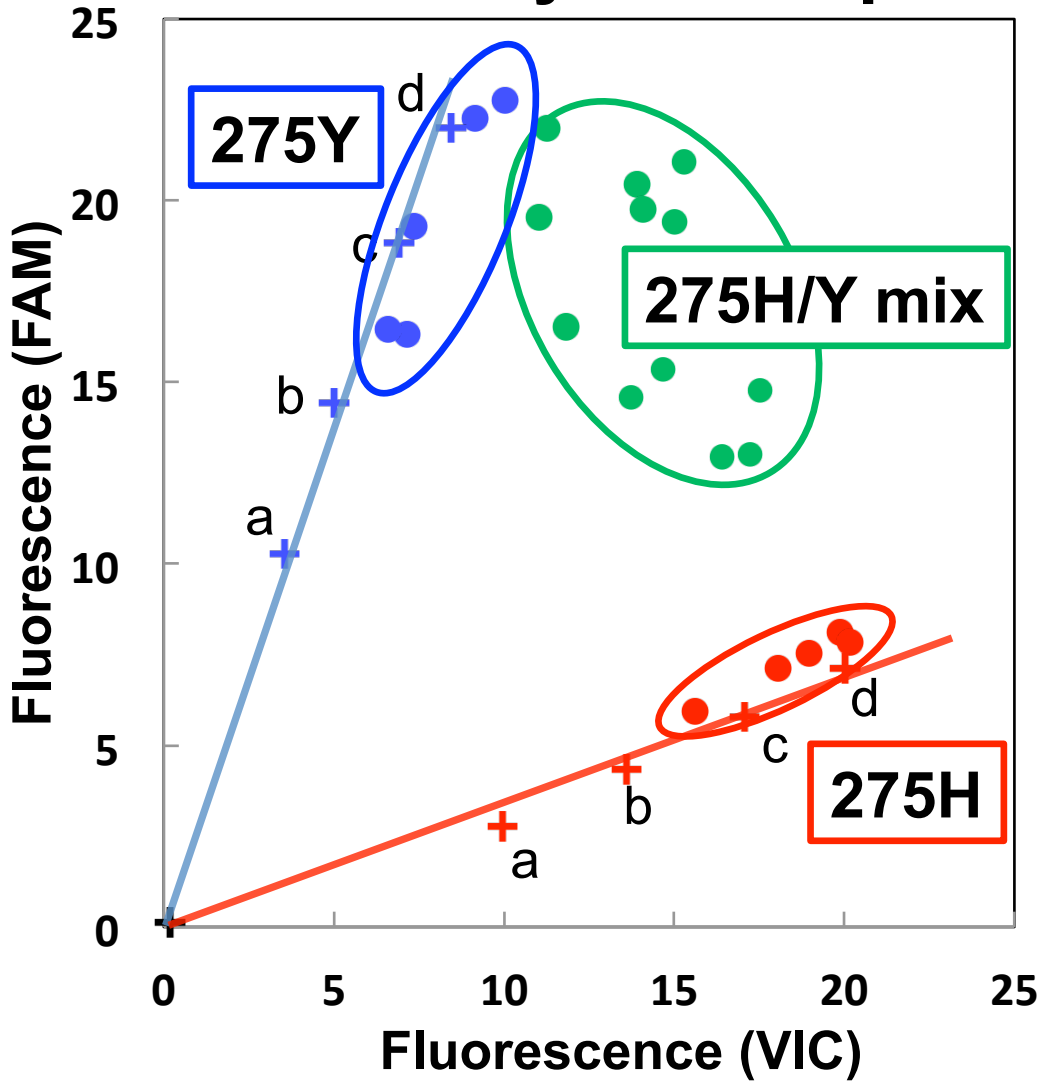


5’-CACTATGAGGAAT-3’

Isolates: without RNA extraction

Clinical specimens: required RNA extraction

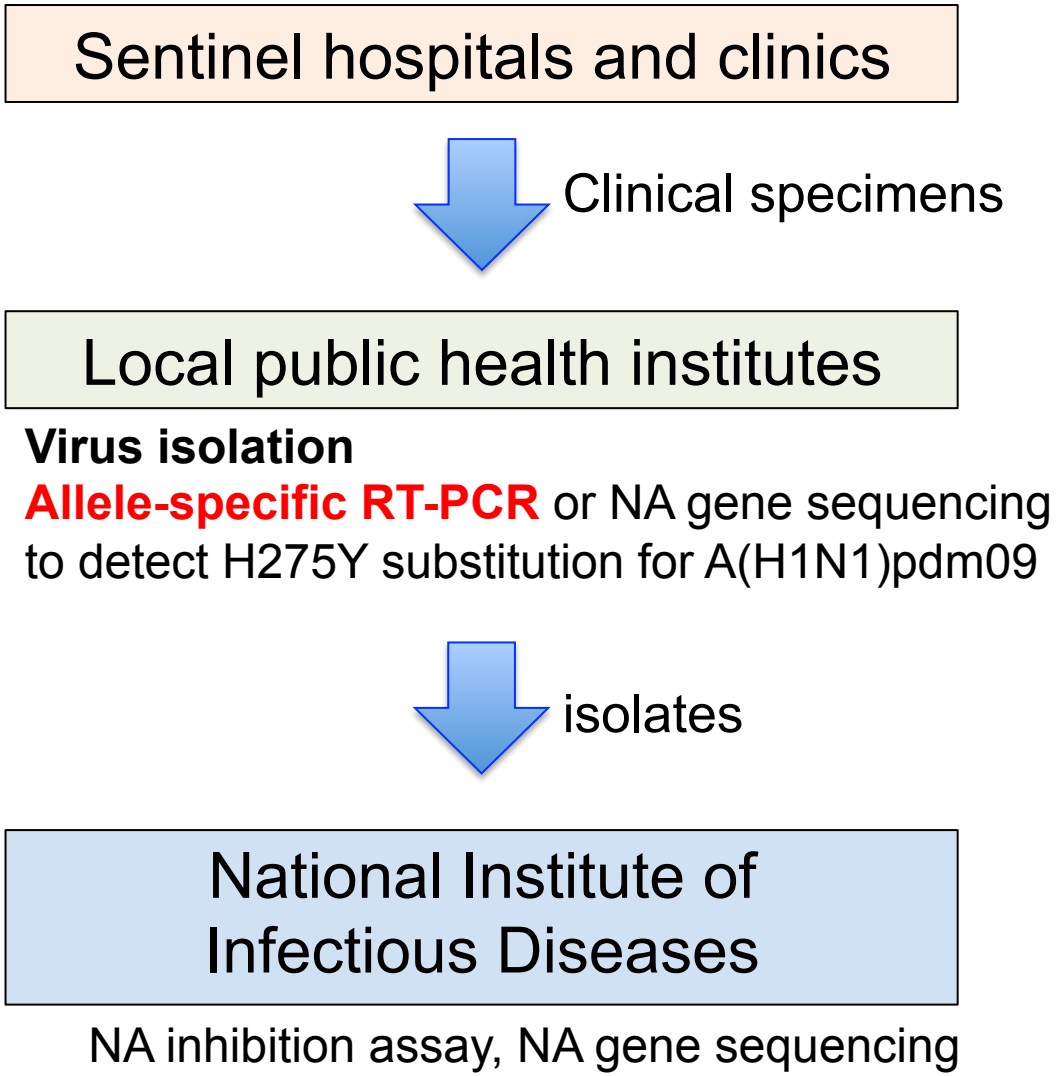
Detection of 275Y and 275H/Y by Allele-specific RT-PCR



- clinical isolates Y275
- clinical isolates H275
- clinical isolates Mix (IC50 >10)
- clinical isolates Mix (IC50 <10)
- \+ Y275 PC (A/Denmark/528/2009)
- \+ H275 PC (A/Denmark/524/2009)
- \+ NC

Positive controls:
a: 3.16 x 10^{1} TCID50/reaction
b: 3.16 x 10^{0} TCID50/reaction
c: 3.16 x 10^{-1} TCID50/reaction
d: 3.16 x 10^{-2} TCID50/reaction

Influenza virus surveillance system in Japan

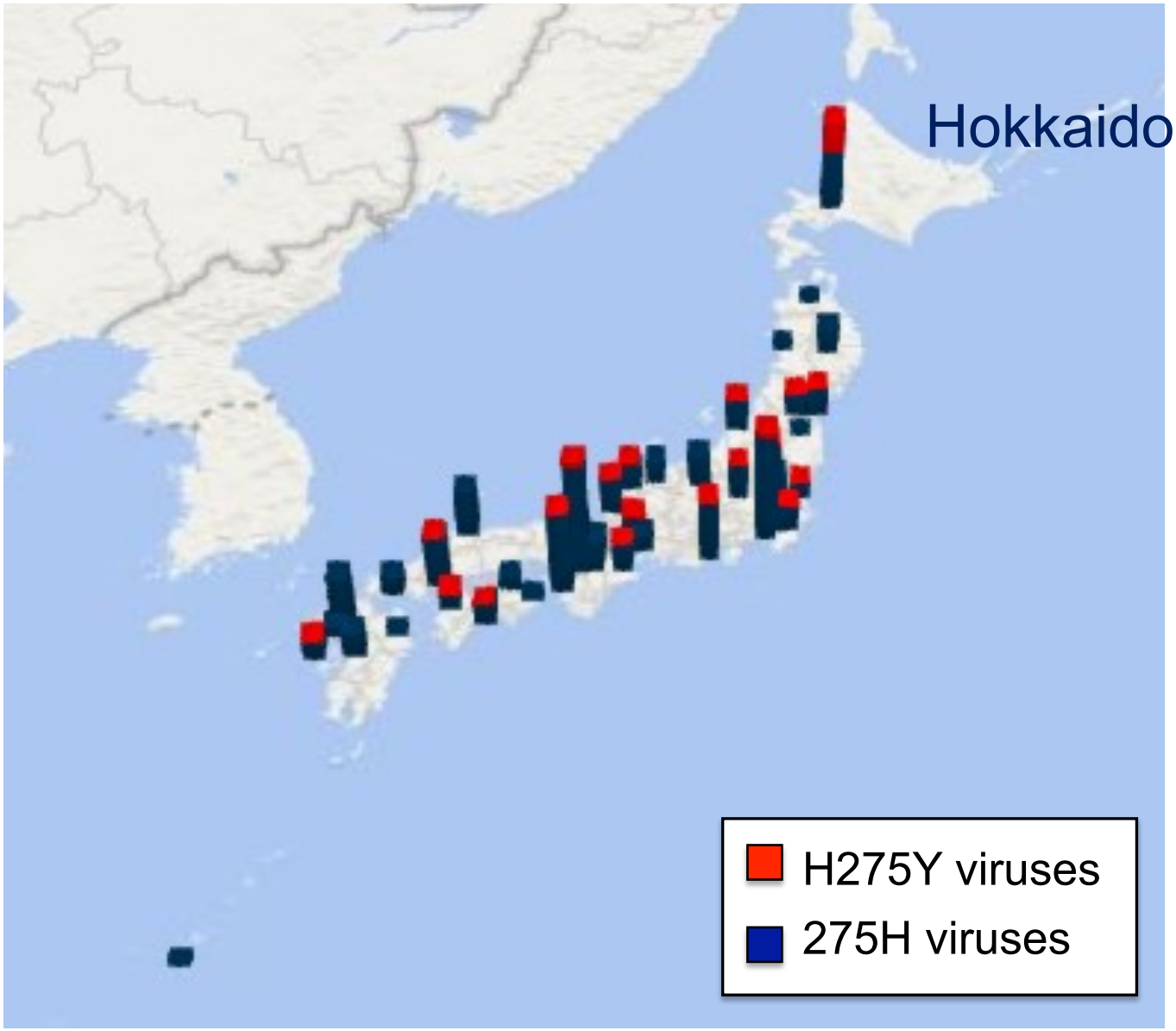


Detection of NA inhibitor-resistant viruses in Japan

Influenza season	A(H1N1)pdm09			A(H3N2)			B		
	No. of viruses detected	No. of viruses tested	Resistant (%)[a]	No. of viruses detected	No. of viruses tested	Resistant (%)[b]	No. of viruses detected	No. of viruses tested	Resistant (%)
2008/2009	9,869	2,168	10 (0.5%)	2,665	195	0	2,039	141	0
2009/2010	22,142	6,005	69 (1.1%)	157	49	0	194	113	0
2010/2011	6,262	3,844	78 (2.0%)	3,865	135	1 (0.7%)	1,848	147	0
2011/2012	19	11	0	5,148	300	1 (0.3%)	2,043	265	0
2012/2013	163	111	2 (1.8%)	5,047	300	0	1,466	337	0
2013/2014	3,493	2,524	105 **(4.2%)**	1,720	285	0	2,956	297	0

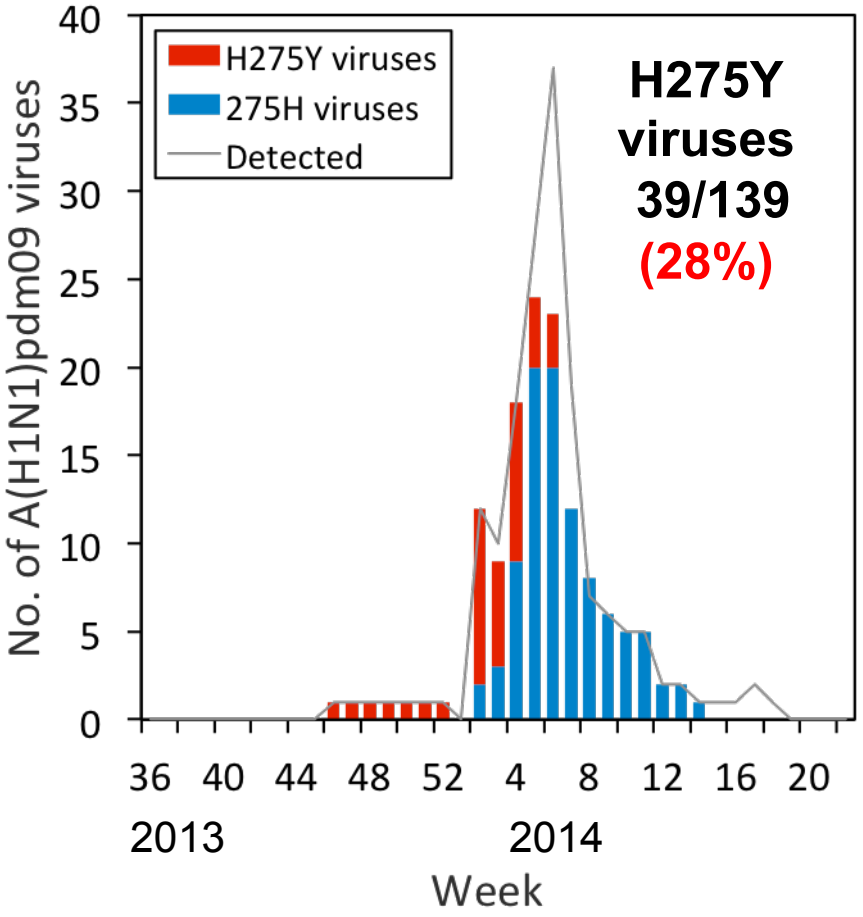
[a] All resistant A(H1N1)pdm09 viruses possessed an H275Y substitution.
[b] All resistant A(H3N2) viruses possessed an R292K substitution.

Distribution of A(H1N1)pdm09 viruses with H275Y substitution in Japan

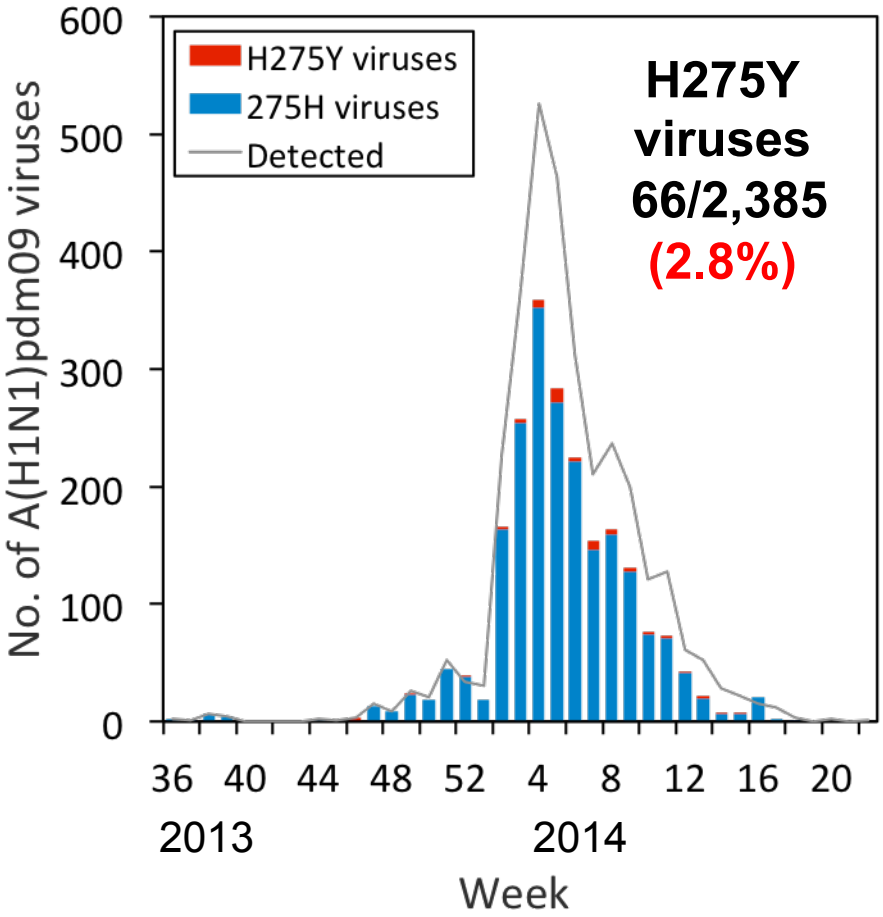


Detection of A(H1N1)pdm09 viruses with the H275Y substitution in Japan

Hokkaido

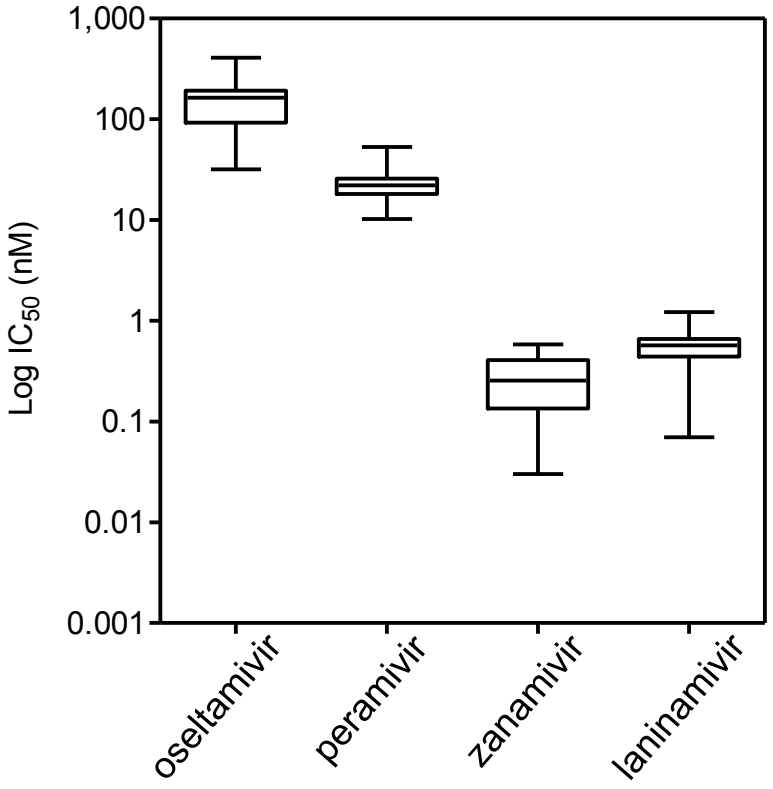


Outside Hokkaido

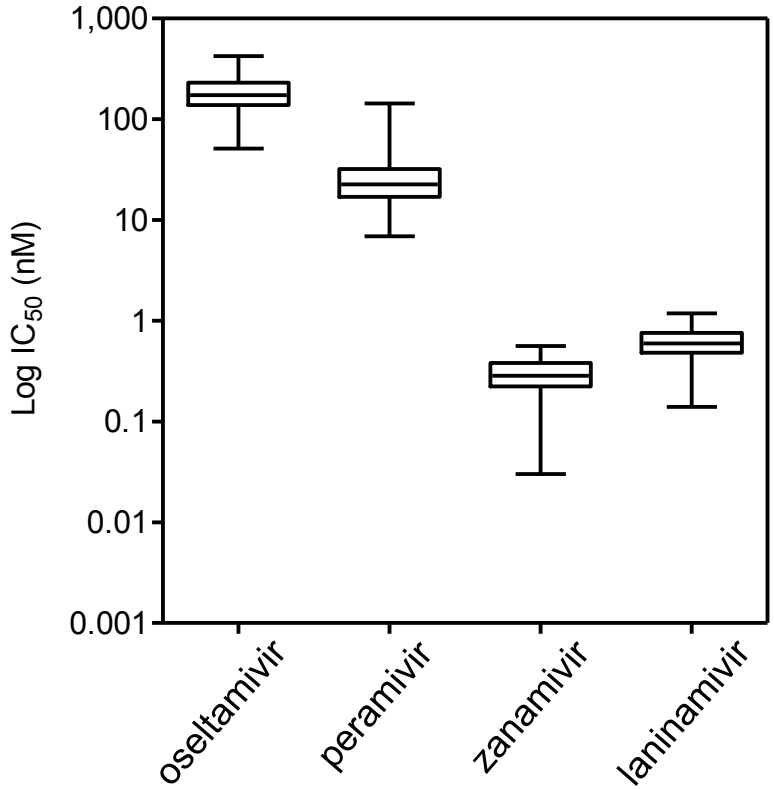


Susceptibility of H275Y mutant viruses to NA inhibitors

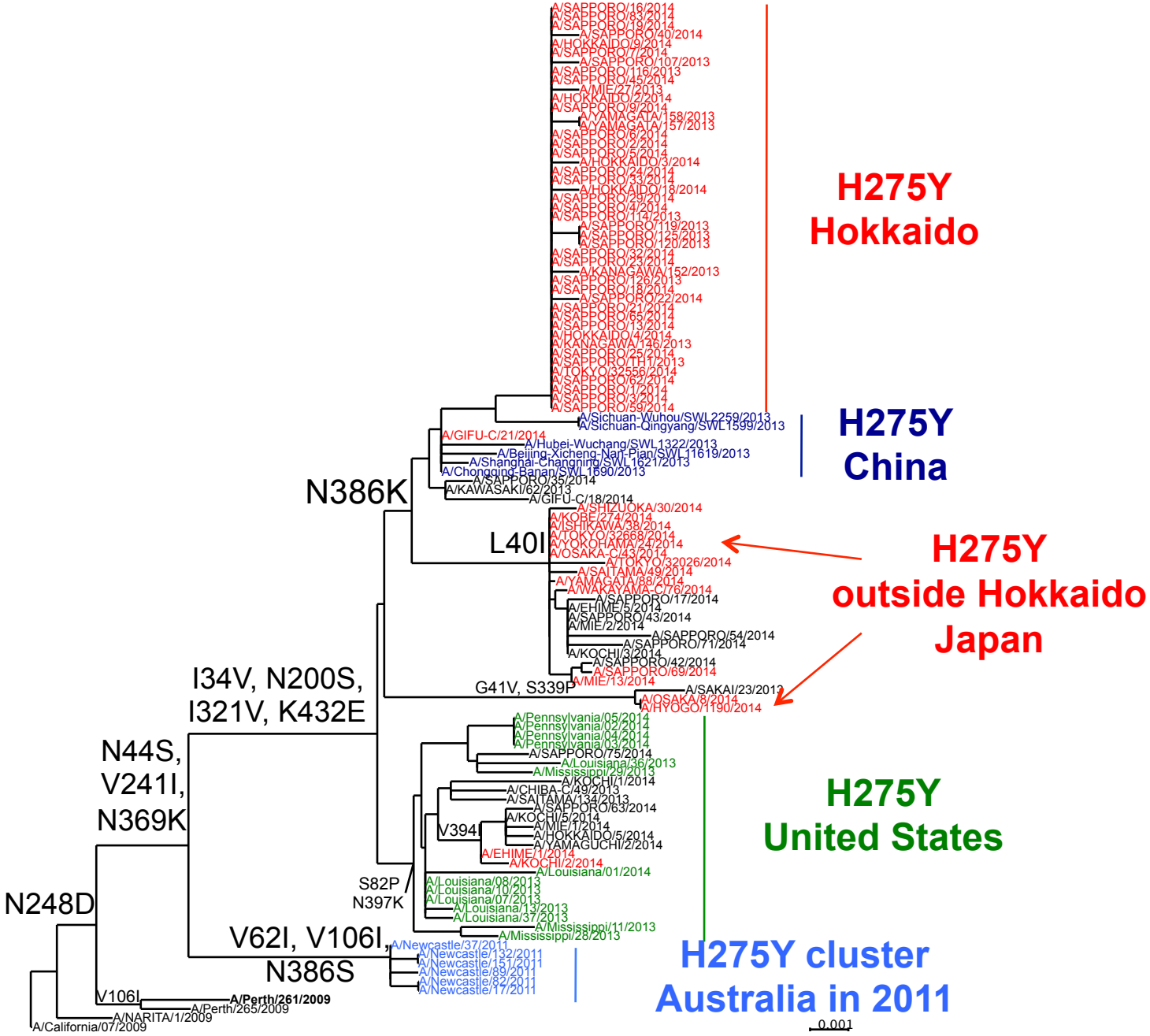
Hokkaido cluster (n = 44)



Sporadic cases (n = 40)



Phylogenetic analysis of NA genes



Characteristic NA amino acids of H275Y mutant viruses

Viral protein	Amino acid position	Reference California/ 07/2009	Newcastle Australia 2011	United States 2013-14	**Hokkaido Japan 2013-14**
NA	241	V	I	I	I
	275	H	Y	Y	Y
	369	N	K	K	K
	386	N	S	N	**K**

Summary

- A large cluster of influenza A(H1N1)pdm09 virus occurred prior to the main influenza epidemic in Hokkaido, Japan. After wild-type virus invaded Hokkaido, the mutant virus disappeared.
- The structure of the mutant NA molecule was less stable than that of the wild-type virus.
- Given that NA inhibitor-resistant viruses in the community have the potential to be readily transmitted among humans, surveillance of these viruses must continue in order to protect public health and aid in clinical management.