

Human Co-Infection with Avian and Seasonal Influenza Viruses, China

Technical Appendix

Technical Appendix Table 1. Summary of PCR primers used to amplify the HA and NA genes

Gene	Primer	Primer sequence (5'→3')	Source
A(RT)	Uni12	AGCAAAAGCAGG	(1)
H7 & H1	Bm-HA-1	TATTCGTCTCAGGGAGCAAAAGCAGGGG	(1)
	Bm-NS-890R	ATATCGTCTCGTATTAGTAGAAACAAGGGTGT	(1)
H7	H7-H1F17	TGGTATTCGCTCTGATTGC	Designed
	H7-H1R523	TAGTCATCTGCGGAATGC	Designed
	H7-H2F420	TAATGGAGCAACCAAGTGC	Designed
	H7-H2R944	GGACATTTTCCAAGTCCCTG	Designed
	H7-H3F834	TGGAGTACAGGTTGATGCCA	Designed
	H7-H3R1434	TTCAAAGCAACCAAGTCCCA	Designed
	H7-H4F1303	TGCTGAACTCTTGGTAGCA	Designed
	H7-H4R1674	AATAGTGCACCGCATGTTTCC	Designed
N9	Bm-N9-1	TATTCGTCTCAGGGAGCAAAAGCAGGGTC	(1)
	Bm-N9-1473R	ATATCGTCTCGTATTAGTAGAAACAAGGGTCTT	(1)
	N9-N1F2	GCGAAAGCAGGGTCAAGAT	Designed
	N9-N1R501	GCTTATCAGGGCGGATACTG	Designed
	N9-N2F416	ATGCTCTCAGCCAAGGAACAA	Designed
	N9-N2R924	TCTATTTGAGCCCTGCCAA	Designed
	N9-N3F857	GCTCATGTTACGGGGAACGA	Designed
	N9-N3R1411	TAGCCCCATCAGGCCAGTT	Designed
H1	H1R2-497	ATGAGGACATGCTGCCGTT	Designed
	H1F2-709	AGTTCAAGCCGGAAATAGC	Designed
	H1R3-1366	AGAACCAACAGTTCGGCAT	Designed
	H1F3-1262	GGGTAAAGAGTTCAACCACCTGG	Designed
	H1R5-1777	CGGAGTAGAAACAAGGGTGTT	Designed
N1	Ba-NA-1	TATTGGTCTCAGGGAGCAAAAGCAGGAGT	(1)
	Ba-NA-1413R	ATATGGTCTCGTATTAGTAGAAACAAGGAGTTTTTT	(1)
	N1R2-489	TTCGATATGGGCTCCTGTC	Designed
	N1F2-421	TGACTCAAGGGGCTTGCTA	Designed
	N1R3-1246	TGCTGAACAAAAGTCCCGCT	Designed
	N1F3-917	GAATCGACCGTGGGTGTCTT	Designed

Gene	Primer	Primer sequence (5'→3')	Source
	N1R6-1457	TGCGTCCGAGTAGAAACAAGGAG	Designed
B(RT)	Uni11	AGCAGAAGCRS	(2)
B-HA	BHF1-1	TTTCTAATATCCACAAAATGAAGGCAA	Designed
	BHR1-234	GACAGTCCGGGCATAGTTTC	Designed
	B-HA-F	ATCCACAAAATGAAGGCAATA	(3)
	B-HA-PR	TGCAGGAGGTCTATATTGG	(3)
	BHF3-778	TACCACAAAAGCGGCAGAATT	Designed
	BHR3-1451	TGCCAATAGATGCTCGTC	Designed
	BHF4-1285	ACCTTCAAAGACTAAGTGGTGC	Designed
	BHR6-1847	GCGCTCAATAACGTTTCTTTG	Designed
B-NA	BNA-F1	CTGAGGCAAATAGGCCAAAAATG	(2)
	BNA-R1	GGGTTTAGAACAGACTCAACC	(2)
	BNA-280F	AAAGCACTCCTAATTAGCCC	(2)
	BNA-467R	GATGCCTCAGCTTGTCTG	(2)
	BNA-537F	GTCCGCATGCCATGATGGTA	(2)
	BNA-871R	GCTGTGTAAGTGTATCTCTACA	(2)
	BNF-861	AAGAATGCACATGCGGATTTGCTA	Designed
	BNR-1270	AAAGGAGTACCAACCAGGTTCTT	Designed
	BNA-1090F	TGGTACTCTCGAACGATGTC	(2)

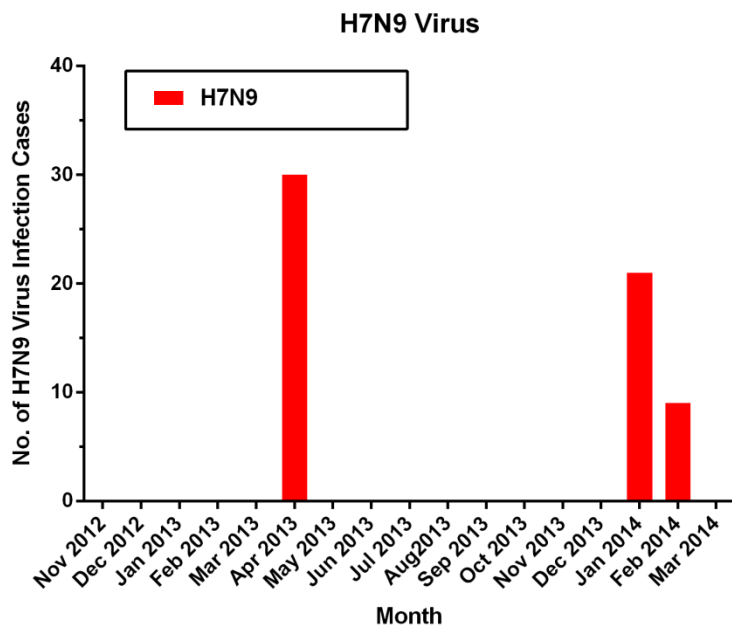
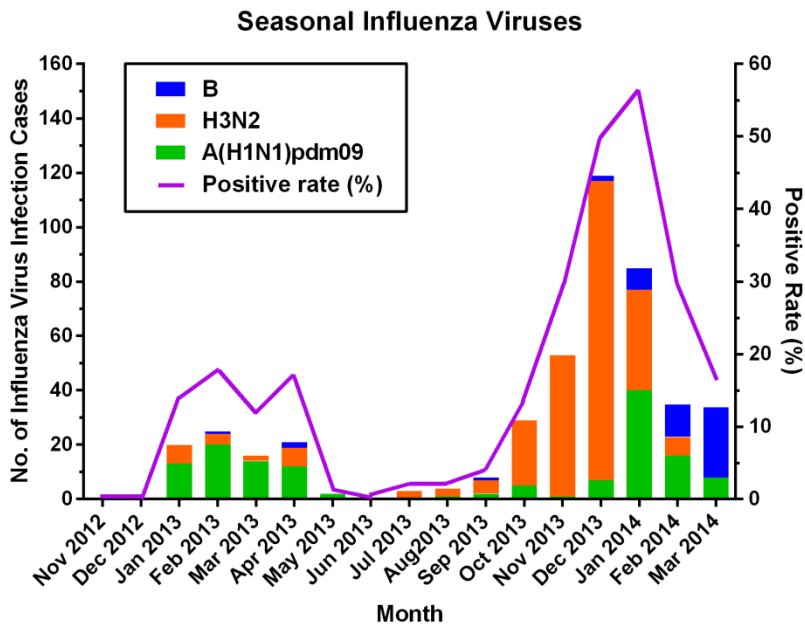
Technical Appendix Table 2. GISAID or GenBank accession numbers of influenza viruses in the construction of the HA and NA phylogenetic trees

Viruses	Segments		Viruses	Segments	
	HA	NA		HA	HA
A/Hangzhou/10-1/2014(H7N9)	EPI509669	EPI509670	A/Chicken/Shanghai/S1053/2013(H7N9)	EPI440685	EPI440684
A/Hangzhou/10-2/2014(H1N1)	EPI509671	EPI511870	A/Environment/Shanghai/S1088/2013(H7N9)	EPI440693	EPI440692
A/Hangzhou/17-1/2014(H7N9)	EPI509672	EPI509673	A/Shanghai/4664T/2013(H7N9)	EPI446962	EPI446965
B/Hangzhou/17-2/2014	EPI509674	EPI509675	A/goose/Jiangsu/K27/2013(H7N9)	EPI442706	
A/Zhejiang/22/2013(H7N9)	EPI477410	EPI477412	A/chicken/Jiangsu/K89/2013(H7N9)	EPI442707	
A/Nanjing/M2/2013(H7N9)	EPI450526	EPI450527	A/duck/Zhejiang/12/2011(H7N3)	JQ906576.1	
A/Zhejiang/DTID-ZJU01/2013(H7N9)	EPI441794	EPI441797	A/duck/Hokkaido/143/2003(H7N1)	AB269694.	
				2	
A/chicken/Zhejiang/DTID-ZJU01/2013(H7N9)	EPI442721	EPI442723	A/duck/Mongolia/867/2002(H7N1)	AB473543.	
				1	
A/Shanghai/1/2013(H7N9)	EPI439486	EPI439487	A/WildBird/Korea/5-77/2005(H7N8)	JX444827.1	
A/Shanghai/2/2013(H7N9)	EPI439502	EPI439500	A/WildBirdFeces/Korea/HDR22/2006(H7N7)	FJ750875.1	
A/Anhui/1/2013(H7N9)	EPI439507	EPI439509	A/WildBirdFeces/Shihwa/21/2006(H7N3)	FJ767723.1	
A/Taiwan/1/2013(H7N9)	EPI445912	EPI445914	A/WildBird/Korea/A330/2009(H7N7)	JN244227.1	
A/Hangzhou/1/2013(H7N9)	EPI440095	EPI440096	A/quail/Thailand/CU-J2882/2009(H7N1)	JX523347.1	
A/Hangzhou/2/2013(H7N9)	EPI442710	EPI442711	A/WildBird/Korea/A9/2011(H7N9)		JN244234.1
A/environment/Hangzhou/34/2	EPI442716	EPI442717	A/mallard/CzechRepublic/13438-29K/2010(H1		JF789602.1

Viruses	Segments		Viruses	Segments	
	HA	NA		HA	HA
013(H7N9)			1N9)		
A/chicken/Hangzhou/48-1/2013(H7N9)	EPI443659	EPI443661	A/goose/CzechRepublic/1848-K9/2009(H7N9)		GU060482. 1
A/chicken/Hangzhou/50-1/2013(H7N9)	EPI454491	EPI454492	A/AnasCrecca/Spain/1460/2008(H7N9)		HQ244409. 1
A/Fujian/1/2013(H7N9)	EPI446746	EPI446747	A/duck/Mongolia/119/2008(H7N9)		AB481213. 1
A/Shanghai/3/2013(H7N9)	EPI443022	EPI443023	A/WildDuck/Korea/SH20-27/2008(H7N9)		JX679164.1
A/Shanghai/4/2013(H7N9)	EPI443025	EPI443026	A/mallard/Sweden/65/2002(H10N9)		CY060360. 1
A/Pigeon/Shanghai/S1069/2013(H7N9)	EPI440701	EPI440700	A/mallard/Netherlands/11/1999(H2N9)		CY122246. 1

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Technical Appendix Figure. Number of seasonal influenza virus infections among patients with influenza-like illness at 2 sentinel hospitals, and positivity rate, by month (upper panel) and number of influenza A(H7N9) virus infections in Hangzhou, China (lower panel). Positivity rate = no. patients with influenza-like illness who were positive for any 1 of the 3 seasonal influenza viruses/no. patients with influenza-like illness tested × 100.