

Table 1 Primer sequences designed for NA subtyping and the expected PCR products

NA subtype	Primer sequence	Product length (bp) ⁺	TM value ⁺⁺
N1-foward	5'-TAGACTGCATGAGGCCTTGCTTCTG-3'	137	78.2~79.2
N1-reverse	5'-CACCGTCTGGCCAAGACCAACCTAC-3'		
N2-foward	5'-ATGTTATCAATTTGCACTTGGGCAG-3'	149	77.1-77.8
N2-reverse	5'-CATGCTATGCACACTTGTTTGGTTC-3'		
N3-foward	5'-ATGATGTCTCTTGGACAAGCAATAG-3'	104	74.8-76.2
N3-reverse	5'-TGGGCATAAACCCAATGTTGGAACC-3'		
N4-foward	5'-AAATCATAACCATCGGTAGTGCGAG-3'	194	76.8-78.6
N4-reverse	5'-TATAGTTGTTCTGCACATTGGTGAC-3'		
N5-foward	5'-CATTTGTGGCATGTGGTCCCACGGA-3'	147	76.6-77.2
N5-reverse	5'-AGGCATTGGGTGAAGATCCTAATGG-3'		
N6-foward	5'-GCAAATAGACCAGTAATCACTAT-3'	153	77.9-78.9
N6-reverse	5'-CCAGGATCTGGGTTTCCTCCTGTTA-3'		
N7-foward	5'-AGCCAAGTATGTTTGGTGGACGAGC-3'	111	79.2-80.3
N7-reverse	5'-TTACGAAAAGTATTGGATTTGTGCC-3'		
N8-foward	5'-TAATGAGTGTAGAAATAGGGCAATC-3'	127	78.9-79.6
N8-reverse	5'-GGAATCAGGGCCCGTTACTCCAA-3'		
N9-foward	5'-ATCGTATTAAACACTGACTGGAGTG-3'	171	78.2-78.9
N9-reverse	5'-ATTCTGTGCTGGAACACATTGATAC-3'		

⁺The expected length of PCR product was calculated according to reference sequences of corresponding NA subtype in NCBI influenza database.

⁺⁺The range of TM value was analyzed by serially diluted DNA or RNA template of different NA subtype in Real-time PCR or Real-time RT-PCR.

Table 2 Quadruplicate primer pools for NA subtyping

Primer pool (NA subtypes)	NA subtypes of AIVs								
	N1	N2	N3	N4	N5	N6	N7	N8	N9
A (N2, N6, N7)		+				+	+		
B (N4, N5, N7, N8)				+	+		+	+	
C (N3, N5, N9)			+		+				+
D (N1, N4, N6, N9)	+			+		+			+

Primer pools were obtained by solving integer program (2) using the IBM CPLEX optimizer. Notice that each column corresponds to a unique amplification pattern, which allows unambiguous identification of the NA subtype present in the sample.