

**S1 Table. Primer Sets, Reagents and Thermocycling Conditions used for two-step Reverse-Transcription Polymerase Chain Reaction (RT-PCR) for Amplification of Influenza B HA and NA genes**

**A. Primer sets**

Gene fragment	Primer	Sequence (5' – 3')	PCR Product Size (bp)
All genes	Buni11W	AGCAGAAGCGS	-
HA-5'	BHAF1u	AGCAGAAGCAGAGCATTTTCTAATATC	1361
	BHAR1341	TTCGTTGTGGAGTTCATCCAT	
HA-3'	BHAF458	AGAAAAGGCACCAGGAGGACCCTA	1391
	BHA2R1	GTAATGGTAACAAGCAAACAAGCA	
NA-5'	BNAF1u	AGCAGAAGCAGAGCATCTTCTCA	1130
	BNAR2	GATGGACAAATCCTCCCTTGATGC	
NA-3'	BNAF2	GCACTCCTAATTAGCCCTCATAGA	1182
	BNAR1487	TAAGGACAATTGTTCAAAC	

Source: London WHO Collaborating Centre, May 2011; WHO information for molecular diagnosis of influenza virus

**B. Reagents and their volumes used for reverse transcription (RT) step.**

Reagent	Vol. (1X), $\mu$ l
Universal Primer (Buni11W)	4.5
Template (RNA)	10.0
Water (Molecular Grade)	12.6
5X First-Strand Buffer*	8.0
0.1M DTT*	2.0
100mM dNTP*	0.9
Superscript <sup>TM</sup> III Reverse Transcriptase*	2.0
Total	40.0

\* Invitrogen<sup>TM</sup>, Life Technologies, USA

**C. Thermocycling conditions for reverse transcription (RT) step.**

Thermocycling steps	Temperature	Cycle	Time (Min)
Denaturation and primer annealing	Mix primer and RNA. Incubate at 65°C. Then chilled on ice.	1	5
Enzyme activation	25°C	1	5
Extension	50°C	1	60
Enzyme deactivation	70°C	1	15

**D. Reagents and their volumes used for polymerase chain reaction (PCR) step.**

Reagent	Vol. (1X), $\mu$ l
10x PCR Buffer (15mM MgCl <sub>2</sub> )*	5.00
dNTP mix (10mM of each)*	1.00
Primer F (10 $\mu$ M)	2.00
Primer R (10 $\mu$ M)	2.00
HotStarTaq <i>Plus</i> DNA Polymerase (250 Units)*	0.25
RNase-free water	35.75
RT product	4.00
Total	50.00

\* QIAGEN<sup>®</sup>, Germany

**E. Thermocycling conditions for polymerase chain reaction (PCR) step.**

Thermocycling steps	Temperature (°C)	Cycle	Time (Minute)
Initial activation	95	1	5
Denaturation	94		0.5
Annealing	58	40	1
Extension	68		1.5
Final Extension	68	1	10