



Protocol for antiviral susceptibility testing by pyrosequencing

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The WHO Collaborating Centre for influenza at CDC Atlanta, United States of America, has made available the protocol, attached, for antiviral susceptibility testing by pyrosequencing of swine influenza A(H1N1).



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SOP Title: Swine-H1N1 M2 and NA pyrosequencing
Effective Date: April 27, 2009

NA: Site H274 (H275 N1 numbering)

RT-PCR primers (20 uM):

Uni-sw-N1-B-F780, GGG GAA GAT TGT YAA ATC AGT YGA

Uni-sw-N1-B-R1273-biot, CWA CCA GAA RCA AGG YCT TAT G

Sequencing primer (100 uM):

Uni-sw-N1-B-F804seq, GYT GAA TGC MCC TAA TT

M2: adamantane susceptibility

RT-PCR primers (20 uM):

sw-M2-F670, AGC TCC AGT GCT GGT CTG AAA G

sw-M2-R900-biot, GAC TCA GGC ACT CCT TCC GTA GAA

Sequencing primer (100 uM):

sw-M2-F747-seq, GCG ATT CAA GTG ATC C

RT-PCR protocol: using Invitrogen Superscript III One step RT-PCR with HiFi Taq system.

	Volume (ul)
2X Reaction Mix	25
Superscript III HiFi Taq	1
Forward primer(20uM)	1
Reverse primer(20uM)	1
Rnase Inhibitor	0.5
Nuclease free water	16.5
Total/sampels	45
Template	5
Final volume	50

PCR Cycling Parameters:

Temp	Length	
50	30min	
94	2min	
94	15sec	45 cycles
55	30sec	
68	60 sec	
68	5min	
4	hold	