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Influenza A(H5) Taqman RT/PCR protocol

Primers/Probe:

SET 1:

RF 1148 5'-GAG-AGG-AAA-TAA-GTG-GAG-TAA-AAT-TGG-A-3'

RF 1149 5'-AAG-ATA-GAC-CAG-CTA-CCA-TGA-TTG-C-3'

RF 1150 5'-6-FAM-TTT-ATT-CAA-CAG-TGG-CGA-GTT-CCC-TAG-CAC-T-TAMRA-3'

SET 2:

RF 1151 5'-GGA-ACT-TAC-CAA-ATA-CTG-TCA-ATT-TAT-TCA-3'

RF 1152 5'-CCA-TAA-AGA-TAG-ACC-AGC-TAC-CAT-GA-3'

RF 1153 5'-6-FAM-TTG-CCA-GTG-CTA-GGG-AAC-TCG-CCA-C-TAMRA-3'

NB: These sets are used in singleplex assays.

Primer/Probe	Stock solution	Work solution
RF 1148	200 pmol/ul	30 pmol/ul
RF 1149	200 pmol/ul	2,5 pmol/ul
RF 1150	200 pmol/ul	10 pmol/ul
RF 1151	200 pmol/ul	40 pmol/ul
RF 1152	200 pmol/ul	2,5 pmol/ul
RF 1153	200 pmol/ul	10 pmol/ul

Nucleic acids are isolated using MagnaPueLC (Roche) using Total NA Isolation Kit and Total NA Serum_Plasma_Blood protocol with 200 µL input, 100 µL output.

RT/PCR 4x TaqMan® Fast Virus 1-Step Master Mix, PN 4444436, Applied Biosystems (LifeTech)
 AmpErase® Uracil N-glycosylase (UNG), PN8080096, Applied Biosystems (LifeTech)

Component	volume/rx
4x TaqMan® Fast Virus 1-Step Master Mix	12,5 µL
UNG (5 U/ul)	0,5 µL
primer/probe mix	1 µL
PCR grade water	16 µL
Extracted nucleic acids	20 µL
total reaction volume	50 µL

Thermal cycling conditions (fast cycling is allowed, not necessary)

Step	Stage	No. of cycles	Temp	Time
Reverse transcription	1	1	50°C	5 min
RT inactivation/initial denaturation	2	1	95°C	20 sec
Amplification	3	45	95°C	3 sec
			60°C	30 sec*

* measure 31 seconds at 7500SDS systems of ABI (or the machine will give an error)

Cover plate with an *optical* adhesive cover. Centrifuge the plate at 1000 rpm for 5 sec.