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Influenza protocol PCR ErasmusMC

- Updates compared to the Jan2011 protocol:
 - All PCRs based on the EZ-mix(ABI) have been deleted since the supply was ended
 - seasonal H1 has been deleted from the standard typing multiplex assay, due to the low incidence of this strain.
 - A new typing RT-PCR (triplex H1mexflu/H3geupdated/PDV) was validated according to ISO15189 guidelines.
 - H3 is updated, since the previous version missed some American H3 strains, which has now been corrected.

Taqman Fast Virus one step Mastermix van Applied Biosystems (P/N 4444432).

The next RT-PCRs will be performed on samples from persons, who are suspected for Influenza like illness:

Influenza A Matrix PCR	FAM-BHQ1 label *
Influenza A H1mexflu PCR	Dragonfly-BHQ2 label (multiplexed with H3 PCR/PDV described below)
Influenza A H3 PCR	FAM-BHQ1 label (multiplexed with H1mexflu PCR described above)*
Influenza B PCR	Dragonfly-BHQ2 label

*(FAM reporter en quencher op NONE), Dragonfly wordt op het TAMRA kanaal gemeten

One step Fast virus mastermix Applied biosystems (P/N 4444432)	
One step mastermix (4x)	12.5 ul
UNG (1 U/ul)	0.5 ul
Primer/probe mix (1 ul/rx)	1 ul
DNase/RNase free H ₂ O	16 ul
Total RT-PCR mix volume	30 ul
Input RNA	20 ul
Total reaction volume	50 ul

One step Fast viral mastermix on ABI7500 or LC480 (Roche)

One step Fast viral mastermix		
2 min	50 °C	
20 sec	95 °C	
3 sec	95 °C	45 cycli
30 sec*	60 °C [#]	

*on ABI7500 is a min. of 31sec detection allowed

acquisition at each cycle.

Primer probe sequences (5'-3') and concentrations per reaction

Influenza A- Matrix	One step Fast viral mastermix	
infa_fwd	cttctRaccgaggctcgaacgta	45 pmol/reaction
infa_probe1	tcaggccccctcaaagccgaga	5 pmol/reaction
infa_probe2	tcaggccccctcaaagccgaaa	5 pmol/reaction
infa_rev	tcttgcttttagccaytccatgag	45 pmol/reaction
Influenza B-NS	One step Fast viral mastermix	
infb-NSfwdB	GRACAACATGACCACAACACAAAT	30 pmol/reaction
infb-NSrevB	CACTCCARAATTCCTGCTTCAAA	45 pmol/reaction
infb-NSprobeB	CGGGAGCAACCAATGCCACCATAAA	5 pmol/reaction
MexFlu H1	One step Fast viral mastermix	
MexFlu-H1-fpr	ggaaagaaatgctggatctggta	30 pmol/reaction
MexFlu-H1-rpr	atgggaggctggtgtttatagc	30 pmol/reaction
MexFlu-H1-prb	tgcaatacaactgtcagacaccaaggg	10 pmol/reaction
Influenza A H3 update2014	One step Fast viral mastermix	
H3HA-fwd	GCAACTGTTACCCTTATGATGT	45 pmol/reaction
H3HA-rev	CCAGTCCAATTGAAGCTTTC	45 pmol/reaction
H3HA-probe	CTTAGGTCACTAGTTGCMTCA	10 pmol/reaction
<u>Optional RT-PCRs</u>		
MexFlu N1	One step Fast viral mastermix	
SwN1-fwd	acatgtgtgtgcaggataactg	30 pmol/reaction
SwN1-rev	tccgaaaatcccactgcatat	30 pmol/reaction
SwN1-probe	atcgaccgtgggtgtctttcaacca	10 pmol/reaction
Influenza A H1 (seasonal)	One step Fast viral mastermix	
H1-fwd1	ccaaagtatgtcaggagtcaaaaat	30 pmol/reaction
H1-fwd2	ccaaagtatgtcaggagtcaaaaat	30 pmol/reaction
H1-rev	ccttcaatgaaaccggcaat	30 pmol/reaction
H1-probe	tggttacaggactaaggaaacatccatcca	5 pmol/reaction