



This protocol has been made available by:

Dr. Suzan Pas
Erasmus Medisch Centrum
Afdeling Viroscience, Na1017
Wytemaweg 80
3015 CN Rotterdam
The Netherlands
Tel: +31 (0)10 7034700
Fax: +31 (0)10 7033441
e-mail: s.pas@erasmusmc.nl

The copyright of this protocol resides with the original author(s).
The original author(s) are responsible for the correctness of the contents of this protocol.
This protocol may be used by microbiological/virological laboratories in the Netherlands.
Distribution of this protocol outside the network and publication in any form are prohibited without obtaining explicit permission of the original author(s), unless the protocol has been published in a journal.

Influenza protocol PCR ErasmusMC

- Updates compared to the Jan2011 protocol:
 - nov 2015 All PCRs based on the EZ-mix(ABI) have been deleted since the supply was ended
 - nov 2015 seasonal H1 has been deleted from the standard typing multiplex assay, due to the low incidence of this strain.
 - nov 2015 A new typing RT-PCR (triplex H1mexflu/H3updated/PDV) was validated according to ISO15189 guidelines.
 - nov 2015 H3 is updated, since the previous version missed some American H3 strains, which has now been corrected.
 - dec 2016 references are added.

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

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

Influenza A Matrix	FAM-BHQ1 label *
Influenza A H1pm2009	Dragonfly-BHQ2 label (multiplexed with H3 PCR/PDV described below)
Influenza A H3update2014	FAM-BHQ1 label (multiplexed with H1mexflu PCR described above)*
Influenza B NS	Dragonfly-BHQ2 label

*(FAM reporter and quencher set at 'NONE'), Dragonfly is measured in the TAMRA channel)

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 (screening)

reference: Hoek RA, Paats MS, Pas SD, Bakker M, Hoogsteden HC, et al. Incidence of viral respiratory pathogens causing exacerbations in adult cystic fibrosis patients. Scand J Infect Dis. 2013 Jan;45(1):65-9. PubMed PMID: 22992129.

infa_fwd	cttctRaccgaggtcgaaacgta	45 pmol/reaction
infa_probe1	tcaggccccctcaaagccgaga	5 pmol/reaction
infa_probe2	tcaggccccctcaaagccgaaa	5 pmol/reaction
infa_rev	tcttgctttagccaytccatgag	45 pmol/reaction

Influenza B-NS (screening)

reference: Hoek RA, Paats MS, Pas SD, Bakker M, Hoogsteden HC, et al. Incidence of viral respiratory pathogens causing exacerbations in adult cystic fibrosis patients. Scand J Infect Dis. 2013 Jan;45(1):65-9. PubMed PMID: 22992129.

infb-NSfwdB	GRACAACATGACCACAACACAAAT	30 pmol/reaction
infb-NSrevB	CACTCCARAATTCCTGCTTCAAA	45 pmol/reaction
infb-NSprobeB	CGGGAGCAACCAATGCCACCATAAA	5 pmol/reaction

Influenza A H1pm2009 (typing)

reference: Van der Vries E, Jonges M, Herfst S, Maaskant J, Van der Linden A, et al. Evaluation of a rapid molecular algorithm for detection of pandemic influenza A (H1N1) 2009 virus and screening for a key oseltamivir resistance (H275Y) substitution in neuraminidase. J Clin Virol. 2010 Jan;47(1):34-7. PubMed PMID: 19857993.

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 (typing)

reference: in house.

H3HA-fwd	GCAACTGTTACCCTTATGATGT	45 pmol/reaction
H3HA-rev	CCAGTCCAATTGAAGCTTTC	45 pmol/reaction
H3HA-probe	CTTAGGTCAGTTGCMTCA	10 pmol/reaction

Optional RT-PCRs

MexFlu N1 (typing)

reference: Van der Vries E, Jonges M, Herfst S, Maaskant J, Van der Linden A, et al. Evaluation of a rapid molecular algorithm for detection of pandemic influenza A (H1N1) 2009 virus and screening for a key oseltamivir resistance (H275Y) substitution in neuraminidase. J Clin Virol. 2010 Jan;47(1):34-7. PubMed PMID: 19857993.

SwN1-fwd	acatgtgtgtgcaggataactg	30 pmol/reaction
SwN1-rev	tccgaaaatcccactgcatat	30 pmol/reaction
SwN1-probe	atcgaccgtgggtgtctttcaacca	10 pmol/reaction

Influenza A H1 (seasonal) (typing)

reference: in house.

H1-fwd1	ccaaagtatgtcaggagtgcaaaat	30 pmol/reaction
H1-fwd2	ccaaagtatgtcaggagtacaaaat	30 pmol/reaction
H1-rev	ccttcaatgaaaccggcaat	30 pmol/reaction
H1-probe	tggttacaggactaaggaacatcccatcca	5 pmol/reaction