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Detection of respiratory viruses or housekeeping genes by qPCR (Taqman) – primers and probes

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GUIDELINES

Follow the reaction and cycling protocol of the qPCR-Taqman mix manufacturer. Attention: after tests, we noticed variation on qPCR efficiency under usage of commercial kits from different brands.

MATERIALS

Set to detect Human Respiratory Syncytial Virus groups A and B [1]

A21 (Forward): 5'- GCTCTTAGCAAAGTCAAGTTGAATGA – 3'

A102 (Reverse): 5'- TGCTCCGTTGCATGGTGTATT – 3'

APB48 (Probe): 5'- FAM/ACACTCAACAAAGATCAACTTCTGTC/TAMRA – 3'

B17 (Forward): 5'- GATGGCTCTTAGCAAAGTCAAGTTAA – 3'

B120 (Reverse): 5'- TGTCAATATTATCTCCTGTACTACGTTGAA – 3'

BPB45 (Probe): 5'- JOE/TGATACATTAAATAAGGATCAGCTGCTGTCATCCA/TAMRA – 3'

Reference [1]: Hu A, Colella M, Tam JS, Rappaport R, Cheng SM. Simultaneous detection, subgrouping, and quantitation of respiratory syncytial virus A and B by real-time PCR. J Clin Microbiol (2003); 41: 149-154. Doi: 10.1128/JCM.41.1.149-154.2003

Set to detect Human Metapneumovirus A and B [2]

HMPV-A (Forward): 5'- GCCGTTAGCTTCAGTCAATTCAA – 3'

HMPV-A (Reverse): 5'- TCCAGCATTGTCTGAAAATTGC – 3'

HMPV-A (Probe): 5'- FAM/CAACATTTAGAAACCTTCT/MGB – 3'

HMPV-B (Forward): 5'- GCTGTCAGCTTCAGTCAATTCAA – 3'

HMPV-B (Reverse): 5'- GTTATCCCTGCATTGTCTGAAAAC – 3'

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HMPV-B (Probe): 5'- FAM/CGCACAAACATTTAGGAATCTTCT/MGB – 3'

Reference [2]: Kuypers J, Wright N, Corey L, Morrow R. Detection and quantification of human metapneumovirus in pediatric specimens by real-time RT-PCR. J of Clin Virol (2005); 33 (4): 299-305. Doi: 10.1016/j.jcv.2004.11.023

Set to detect Influenza virus A [3]

FLUA (Forward): 5'- GACCRATCCTGTACCTCTGAC – 3'

FLUA (Reserve): 5'- AGGGCATTYTGACAAAKCGTCTA – 3'

FLUA (Probe): 5'- FAM/TGCAGTCCTCGCTCACTGGGCACG/BHQ1 – 3'

Reference [3]: CDC (2009) Protocol of real time RT-PCR for influenza A. Available: <http://www.who.int/csr/resources/publications/swineflu/realtimptpcr/en/index.html>.

Set to detect Influenza virus B [4]

INFB-1 (Forward): 5'- AAATACGGTGGATTAAATAAAAGCAA – 3'

INFB-2 (Reserve): 5'- CCAGCAATAGCTCCGAAGAAA – 3'

INFB (Probe): 5'- JOE/CACCCATATTGGGCAATTCCTATGGC/TAMRA – 3'

Reference [4]: van Elden LJ, Nijhuis M, Schipper P, Schuurman R, van Loon AM. Simultaneous detection of influenza viruses A and B using real-time quantitative PCR. J Clin Microbiol (2001); 39: 196-200. Doi: 10.1128/JCM.39.1.196-200.2001

Set to detect Parainfluenza virus 1 [5]

PIV1 (Forward): 5'- ACAGATGAAATTTTCAAGTGCTACTTTAGT – 3'

PIV1 (Reserve): 5'- GCCTCTTTTAATGCCATATTATCATTAGA – 3'

PIV1 (Probe): 5'- FAM/ATGGTAATAAATCGACTCGCT/MGB – 3'

Reference [5]: Kuypers J, Wright N, Ferrenberg J, Huang ML, Cent A, et al. Comparison of real-time PCR assays with fluorescent-antibody assays for diagnosis of respiratory virus infections in children. J of Clin Microbiol (2006); 44 (7): 2382-2388. Doi: 10.1128/JCM.00216-06

Set to detect Parainfluenza virus 3 [6]

PIV3 (Forward): 5'- CTCGAGGTTGTCAGGATATAG – 3'

PIV3 (Reserve): 5'- CTTGGGAGTTGAACACAGTT – 3'

PIV3 (Probe): 5'- FAM/AATAACTGTAACTCAGACTTGGTACCTGACTT/TAMRA – 3'

Reference [6]: Garbino J, Gerbase MW, Wunderli W, Deffernez C, Thomas Y, et al. Lower respiratory viral illnesses: improved diagnosis by molecular methods and clinical impact. *Am J Respir Crit Care Med* (2004); 170: 1197-1203. Doi: 10.1164/rccm.200406-781OC

Set to detect Human Bocavirus [7]

BOV(Forward): 5'- GCACAGCCACGTGACGAA – 3'

BOV (Reserve): 5'- TGGACTCCCTTTTCTTTTGTAGGA – 3'

BOV (Probe): 5'- JOE/TGAGCTCAGGGAATATGAAAGACAAGCATCG/TAMRA – 3'

Reference [7]: Neske F, Blessing K, Tollmann F, Schubert J, Rethwilm A, et al. Real-time PCR for diagnosis of human bocavirus infections and phylogenetic analysis. *J Clin Microbiol* (2007); 45: 2116-2122. Doi 10.1128/JCM.00027-07

Set to detect Adenovirus [8]

BOV(Forward): 5'- GCCACGGTGGGGTTTCTAAACTT – 3'

BOV (Reserve): 5'- GCCCCAGTGGTCTTACATGCACATC – 3'

BOV (Probe): 5'- FAM/TGCACCAGACCCGGGCTCAGGTACTCCGA/TAMRA – 3'

Reference [8]: Heim A, Ebnet C, Harste G, Pring-Akerblom P. Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *J Med Virol* (2003); 70: 228-239. Doi:10.1002/jmv.10382

Set to detect β -Actin [9]

ACT (Forward): 5'- CCCAGCCATGTACGTTGCTA – 3'

ACT (Reserve): 5'- TCACCGGAGTCCATCACGAT – 3'

ACT (Probe): 5'- VIC/ACGCCTCTGGCCGTACCACTGG/TAMRA – 3'

Reference [9]: Nystrom K, Biller M, Grahn A, Lindh M, Larson G, et al. Real time PCR for monitoring regulation of host gene expression in herpes simplex virus type 1-infected human diploid cells. *J Virol Methods* (2004); 118: 83-94. Doi: 10.1016/j.jviromet.2004.01.019

Set to detect RNaseP [3]

ACT (Forward): 5'- AGATTTGGACCTGCGAGCG – 3'

ACT (Reverse): 5'- GAGCGGCTGTCTCCACAAGT – 3'

ACT (Probe): 5'- FAM/TTCTGACCTGAAGGCTCTGCGCG/BHQ1 – 3'

Reference [3]: CDC (2009) Protocol of real time RT-PCR for influenza A. Available: <http://www.who.int/csr/resources/publications/swineflu/realtimeptpcr/en/index.html>.

- 1** Detection of hRSV-A/B; hMPV-A/B (Duplex format of qPCR-Taqman). For final volume reaction of 15µl, add 3µL cDNA, 0.5µL Primer Forward A (10pM), 0.5µL Primer Reverse A (10pM), 0.5µL Primer Forward B (10pM), 0.5µL Primer Reverse B (10pM), 0.25µL Probe A (10pM), 0.25µL probe B (10pM).
- 2** Detection of FLU-A, FLU-B, PIV-1, PIV-3. For final volume reaction of 15µl, add 3µL cDNA, 0.5µL Primer Forward (10pM), 0.5µL Primer Reverse (10pM), 0.25µL Probe (10pM)
- 3** Detection of hBoV, AdV, β -Actin and RNaseP. For final volume reaction of 15µl, add 3µL cDNA, 0.25µL Primer Forward (10pM), 0.25µL Primer Reverse (10pM), 0.125µL Probe (10pM)