2019

Working

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

PLOS One

José Luiz-Proença Modena[1], Flavia Escremim de Paula[2], Eurico Arruda[2]

[1]University of Campinas - SP, [2]School of Medicine University of São Paulo

dx.doi.org/10.17504/protocols.io.w8yfhxw

Talita B. Gagliardi
University of Maryland, College Park

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0217744

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Matsuno AK, Gagliardi TB, Paula FE, Luna LKS, Jesus BLS, Stein RT, Aragon DC, Carlotti APCP, Arruda E (2019) Human coronavirus alone or in co-infection with rhinovirus C is a risk factor for severe respiratory disease and admission to the pediatric intensive care unit: A one-year study in Southeast Brazil. PLoS ONE 14(6): e0217744. doi: 10.1371/journal.pone.0217744

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 TEXT

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

A21 (Forward): 5'- GCTCTTAGCAAAGTCAAGTTGAATGA – 3'
A102 (Reserve): 5'- TGCTCCGTTGCATGGTGTATT – 3'
APB48 (Probe): 5'- FAM/ACACTCAACAAAGATCAACTTCTGTC/TAMRA – 3'

B17 (Forward): 5'- GATGGCTCTTAGCAAAGTCAAGTTAA – 3'
B120 (Reserve): 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 (Reserve): 5'- TCCAGCATTGTCTGAAAATTGC – 3'
HMPV-A (Probe): 5'- FAM/CAACATTTAGAAACCTTCT/MGB – 3'

HMPV-B (Forward): 5'- GCTGTCAGCTTCAGTCAATTCAA – 3'
HMPV-B (Reserve): 5'- GTTATCCCTGCATTGTCTGAAAACT – 3'
HMPV-B (Probe): 5'- FAM/CGCACAACATTTAGGAATCTTCT/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'- GACCRATCCTGTCACCTCTGAC – 3'
FLUA (Reserve): 5'- AGGGCATTYTGGACAAAKCGTCTA – 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/realtimeptpcr/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/CACCCATATTGGGCAATTTCCTATGGC/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/AATAACTGTAAACTCAGACTTGGTACCTGACTT/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 (Reserve): 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 Foward A (10pM), 0.5µL Primer Reverse A (10pM), 0.5µL Primer Foward 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 Foward (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 Foward (10pM), 0.25µL Primer Reverse (10pM), 0.125µL Probe (10pM)