Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement Material

Diagnose using the RespiFinderSmart22kit

Extracted nucleic acid samples from patients were tested for viruses and bacteria using the RespiFinderSmart22kit (PathoFinder BV, Netherlands) and the LightCycler 480 real-time PCR system in accordance with manufacturer instructions[1]. Samples were analyzed for 22 pathogens (18 viruses and 4 bacteria): influenza A (IFV-A), influenza A (H1N1) pdm09 virus [IFV-A (H1N1) pdm09], influenza B (IFV-B), respiratory syncytial virus type A (RSV-A), respiratory syncytial virus type B (RSV-B), human metapneumovirus (hMPV), parainfluenza virus type 1 (PIV-1), parainfluenza virus type 2 (PIV-2), parainfluenza virus type 3 (PIV-3), parainfluenza virus type 4 (PIV-4), rhinovirus/enterovirus (RV/EV), human coronavirus NL63 (HCoV-NL63), human coronavirus HKU1 (HCoV-HKU1), human coronavirus 229E (HCoV-229E), human coronavirus OC43 (HCoV-OC43), human bocavirus (HBoV), adenovirus (AdV), Bordetella pertussis (B. pert), Chlamydia pneumoniae (C. pneu), Legionella pneumophila (L. pneu), and Mycoplasma pneumoniae (M. pneu).


Several one-step rRT-PCR assays were developed using the One Step PrimeScript™ RT-PCR kit (TaKaRa, Japan). Each 25 µl reaction mixture contained 12.5 µl of 2× Master Mix, 0.5 µl of reverse transcriptase/Taq DNA polymerase mixture, 5 µl of RNA, 400 nM concentrations of forward primer and reverse primer, and 200nM of probe. Thermal cycling included 42°C for 5 minutes, followed by 95°C for 10 s and then 40 cycles of 95°C for 10 s and 60°C for 45 seconds.

Primers and probes described as below:

Set I (ORF1ab)
- Forward primer: CCCTGTGGGTTTTACACTTAA
- Reverse primer: ACGATTGTGCATCAGCTGA
- Fluorescent probe (P): 5’ - the FAM - CCGTCTGCGGTATGTGGAAAGGTTATGG - BHQ1-3’

Set II (N)
- Forward primer: GGGGAACCTCTCCTGCTAGAAT
- Reverse primer: CAGACATTTTGCTCTCAAGCTG
- Fluorescent probe: 5’-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3’

Reference