

## Supplementary Appendix

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

Supplement to: Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020;382:727-33. DOI: 10.1056/NEJMoa2001017

## 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*).

### Real-Time RT-PCR Assay for Screening of 2019-nCoV Infection.

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: CCCTGTGGGTTTTACTTAA;

Reverse primer: ACGATTGTGCATCAGCTGA;

Fluorescent probe (P): 5' - the FAM - CCGTCTGCGGTATGTGGAAAGGTTATGG - BHQ1-3'.

#### Set II (N)

Forward primer : GGGGAACTTCTCCTGCTAGAAT;

Reverse primer: CAGACATTTTGCTCTCAAGCTG;

Fluorescent probe: 5' -FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3'.

### Reference

1. Liu G, Li H, Zhao S, Lu R, Niu P, Tan W. Viral and Bacterial Etiology of Acute Febrile Respiratory Syndrome among Patients in Qinghai, China. *Biomed Environ Sci*. 2019 Jun;32(6):438-445.