EXPERIMENTAL PROCEDURES

Respiratory pathogens nucleic acid detection

Total extracted nucleic acids from respiratory samples were eluted in 50 µl of elution buffer (NucliSens Buffer 3, BioMérieux, France) and immediately subjected to nucleic acid amplification. The FTD Respiratory Pathogens 21 plus assay (Fast Track Diagnostics, Luxembourg) is a six multiplex RT-PCR reaction which allows the detection of: influenza A, influenza A (H1N1), influenza B, coronaviruses NL63, 229E, OC43 and HKU1, parainfluenza 1, 2, 3 and 4, rhinovirus, respiratory syncytial viruses A and B, human metapneumovirus A and B, adenovirus, enterovirus, parechovirus, bocavirus, *Mycoplasma pneumoniae, Streptococcus pneumoniae, Haemophillus influenza, Staphylococcus aureus* and *Chlamydia pneumoniae*. Ten microliters of extracted nucleic acids were added in each multiplex tube of the assay and amplification/detection was performed in a CFX96 real-time PCR machine (Bio-Rad, USA).

Pathogen discovery protocol

The samples were first pretreated in order to limit human genomic DNA contaminations as following: 200 µl of NA or PE were centrifuged, filtered through a 0.22 µm disk filter (Millex GV, Millipore, USA) and then ultracentrifuged at 729,600 g for 90 min. The resulting pellet was dissolved in 200 µl of molecular-biology-grade water. Seventy units of DNase I (1U/µl, Promega, USA) were added, and the samples were incubated for 2 h at 37°C. Nucleic acids were extracted with easyMAG and eluted in 50 µl of elution buffer. Extracted DNA and RNA were amplified by random RT-PCR. Reverse transcription was performed at 42°C for 60 min with the FR26RV-N primer at 10µM using ImProm-II reverse transcriptase (Promega, USA). Then 3’-5’ exo$^-$ Klenow DNA polymerase (New England Biolabs, USA) was added and the reaction was incubated at 37°C for 1 h. Five microliters of each reaction mix were used as a template for the subsequent PCR. Amplification was performed in 50µl using Expand High Fidelity PCR System (Invitrogen, Germany) with 40 pmol of FR20RV primer. After 5 min at 95°C, 40 cycles of amplification (95°C for 30 s, 62°C for 1 min, and 68°C for 2 min) were accomplished. The amplification products were purified with QIAquick PCR Purification Kit (Qiagen, USA) and cloned into the vector pCR-2.1 in electro-competent *E. coli* TOP-10 according to the manufacturer’s instructions (TOPO TA Cloning Kit, Invitrogen, Germany). Ninety-six clones per sample were sent to GATC Biotech (Switzerland) for Sanger sequencing.