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## Supplementary information

# Virological assessment of hospitalized patients with COVID-2019

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# Virological assessment of hospitalized cases of coronavirus disease 2019

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## Supplementary Methods

## Table of content

## 1. Supplementary description of RNA extraction and RT-PCR methods

### a. RNA extraction RT-PCR for SARS-CoV-2 and quantification

RNA was extracted from clinical samples by using the viral RNA mini kit (Qiagen, Hilden Germany) following the manufacturer´s instructions. Sputum samples were diluted 1:3 and stool samples 1:5 in phosphate-buffered saline, mixed, and briefly centrifuged before RNA extraction. Real-time RT-PCR for SARS-CoV-2 was performed on RNA extracts with RT-PCR targets in the E- and RdRp genes, as described (Corman, Landt et al. 2020). Both laboratories used a pre-formulated oligonucleotide mixture (Tib-Molbiol, Berlin, Germany) to make laboratory procedures more reproducible. In cases of PCR inhibition, samples were re-tested in 1:10 and 1:100 dilutions.

### b. RT-PCR for subgenomic RNA for SARS-CoV-2

Testing for sgRNA used a leader-specific primer, as well as primers and probes targeting sequences downstream of the start codons of the E gene, as described (Corman, Landt et al. 2020). In addition
to real-time RT-PCR readouts, RT-PCR products for sgRNAs were analyzed on agarose gel and Sanger sequenced (**Figure S1**). The oligonucleotide sequence of the leader specific primer was as follows: sgLeadSARSCoV2-F; CGATCTCTTGTAGATCTGTTCTC. The sgRNA RT-PCR assay used the Superscript III one-step RT-PCR system with Platinum Taq Polymerase (Invitrogen, Darmstadt, Germany) with 400 nM concentrations of each of the primers, as well as 200 nM of probe. Thermal cycling involved 10 min at 50 °C for reverse transcription, followed by 3 min at 95 °C and 45 cycles of 10 s at 95 °C, 15 s at 56 °C, and 5 s at 72 °C.

### c. RT-PCR for other respiratory viruses

All patients (one sputum and one oro- or nasopharyngeal throat swab from each) were tested for other respiratory viruses using LightMix-Modular Assays (Roche, Penzberg, Germany). These PCR assays test for presence of HCoV-HKU1, -OC43, -NL63, -229E; Influenza virus A and B, Rhinovirus, Enterovirus, Respiratory syncytial virus, Human Parainfluenza virus 1-4, Human metapneumovirus, Adenovirus, and Human bocavirus RNA or DNA.

## 2. Supplementary description of cell culture and antibody detection methods

### a. Virus isolation

Vero E6 (ATCC CRL-1586) cells were seeded on a 24-well plate at 3.5 × 10^5 cells/mL in Dulbecco modified Eagle medium (DMEM) containing 1% sodium pyruvate, 1% nonessential amino acids, 1% l-glutamine, and 10% fetal calf serum (FCS; all Gibco, Darmstadt, Germany) one day prior to infection. Sputum and swabs from patients were diluted in 2 ml OptiPro serum-free medium (Gibco) to reduce viscosity and improve pipetting. Stool samples were dissolved in 3 mL OptiPro medium. Stool samples were centrifuged for 30 min at 300 x g and 4°C. The supernatant of the stool samples was further diluted (1:5 and 1:50) in OptiPro medium and passed through a 0.45 µm-sized filter.

Vero E6 cells were inoculated with 100 µl processed patient sample (100 ul) at 37°C for 1 hour. Then, cells were washed ones with phosphate-buffered saline (Gibco) and supplied with 500 µl DMEM composite as described above, except for a reduced FCS content of 2% and 1% amphotericin B. Cells were controlled daily for cytopathogenic effects for 6 days. Every 2 days or upon observation of cytopathogenic effects, 50 µl of cell culture supernatant was subjected to viral RNA extraction and SARS-CoV-2 specific real-time RT-PCR using the SARS-2-CoV E assay. Supernatants of positively tested wells were harvested, centrifuged at 200 × g for 3 min to remove cell debris and diluted 1:2 in OptiPro containing 0.5% gelatin for storage.

### b. Recombinant SARS-CoV-2 spike protein-based immunofluorescence test

VeroB4 cells were transfected with pCG1-SARS-CoV-2 Spike (kindly provided by Stefan Pöhlmann, DPZ, Göttingen, Germany) or with previously described pCG1-Spike encoding plasmids representing HCoV-NL63, -229E, -OC43, -HKU1.(Corman, Muller et al. 2012) For transfection, Fugene HD (Roche, Penzberg, Germany) was used in a Fugene to DNA ratio of 3:1. After 24 hours, the transfected VeroB4 cells were harvested and resuspended in DMEM/10% FCS to achieve a cell density of $2.5x10^5$ cells/ml. 50 µlof the cell suspension was applied to each incubation field of a multitest cover slide (Dunn Labortechnik, Asbach, Germany). The multitest cover slides were incubated for 6 hours before they were washed with PBS and fixed with ice-cold acetone/methanol (ratio 1:1) for 10 minutes. For the immunofluorescence test, the incubation fields were blocked by applying 5% non-fat dry milk in PBS/0.1% Tween for 30 minutes. Heat-inactivated patient sera (56°C, 30 minutes) were diluted in EUROIMMUN sample buffer (serial dilutions 1:10, 1:100, 1:1000, 1:10000) and 30 µl of the dilution was applied per incubation field. For IgM detection, IgG were depleted before applying sera to immunofluorescence tests. After 1 hour at room temperature, cover

slides were washed 3 times for 5 minutes with PBS/0.1% Tween. Secondary detection was done using a 1:200 dilution of a goat-anti human IgG-Alexa488 (Dianova). After 30 minutes at room temperature, slides were washed 3 times for 5 minutes and rinsed with water. Slides were mounted using DAPI prolonged mounting medium (FisherScientific, Schwerte, Germany).

### c. Plaque reduction neutralization test (PRNT) for SARS-CoV 2

Plaque reduction neutralization tests were done as described for MERS-CoV (Drosten, Meyer et al. 2014) VeroE6 cells ($4x10^5$ cell/ml) were seeded in 24-well plates and incubated overnight. Prior to PRNT patient sera were heat-inactivated at 56°C for 30 minutes. For each dilution step (duplicate), patient sera were diluted in 200 µl OptiPro and mixed 1:1 with 200 µl virus solution containing 100 plaque forming units. The 400 µl serum-virus solution was vortexed gently and incubated at 37°C for 1 hour. Each 24-well was incubated with 200 µl serum-virus solution. After 1 hour at 37°C the supernatants were discarded, the cells were washed once with PBS and supplemented with 1.2% Avicel solution in DMEM. After 3 days at 37°C, the supernatants were removed and the 24-well plates were fixed and inactivated using a 6% formaldehyde/PBS solution and stained with crystal violet as described (Herzog, Drosten et al. 2008).

## 3. Supplementary references


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