Supplementary Appendix

Title: Prolonged (6 months) Shedding of Middle East Respiratory Syndrome Coronavirus RNA in Sputum of a Lymphoma Patient

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METHOD

Clinical specimens

Clinical specimens, including sputum, throat swabs, and serums, were collected every 3-4 days from the time of admission to the study hospitals. Nasopharyngeal and throat swabs were collected using UTMTM Kit containing 3 mL of viral transport media (Copan Diagnostic Inc., Murrieta, CA). Specimens were stored at -80°C until testing.

MERS-CoV viral load assay

RNA was extracted using the QIAamp viral RNA mini kit (QIAGEN, Valencia, CA). To measure the viral load, we performed one-step multiplex quantitative rRT-PCR using PowerChekTM MERS (upE & ORF1a) Real-time PCR kit (Kogenebiotech, Seoul, South Korea). All assays were performed in duplicate with ViiATM 7 Real-time PCR system (Applied Biosystems®, Grand Island, NY). Positive controls of known concentration of 1.0×10^5 copies per μL were purchased from the European Virus Archives.

Viral culture

We inoculated Vero cells, Vero E6 cells, and Caco-II cells with all specimens showing positive results from rRT-PCR and cultured at 37°C in a 5% CO_2 atmosphere and checked daily for cytopathic changes. Culture positive was defined that adequate cytopathic effect was observed, and the passage of supernatant onto new cells induced the cytopathic effect again. If the cytopathic effect was observed, virus proliferation was confirmed by rRT-PCR for culture supernatant. If no cytopathic effect was observed until seven days after inoculation, cells were harvested by cell scraper and centrifuged, and the supernatant was inoculated onto new cells. If the cytopathic effect was not observed until the second passage and Ct value did not decline in rRT-PCT,

the culture was considered to be negative. If the virus was cultured, the full-length genome sequence of the isolate was analyzed as previously described [1]. All experiments were done at a BSL-3 facility in Seoul National University Hospital permitted by the Korean Centers for Disease Control and Prevention.

Subgenomic mRNA assay

The MERS-CoV sub-genomic mRNA was detected using AccuPower RT-PCR PreMix (Binder Inc., Alameda, CA, USA). PCR primers were designed to detect sub-genomic mRNA that codes for the spike (S) (433 bp) and nucleocapsid (N) (662 bp) proteins, as previously described [2]. The forward primer was elaborated from the leader sequence, and backward primers of 50 untranslated regions (UTR)-S and 50 UTR-N were from gene sequences coding for proteins S and N, respectively. Sub-genomic mRNA was sequenced using a DNA Engine Tetrad 2 Peltier Thermal Cycler (Bio-Rad) and the ABI BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Grand Island, NY, USA). If the sequences included the leader sequence and were consistent with the MERS-CoV genome by 98% using Basic Local Alignment Search Tool (BLAST) software, they were confirmed as sub-genomic mRNA.

Direct sequencing for S protein

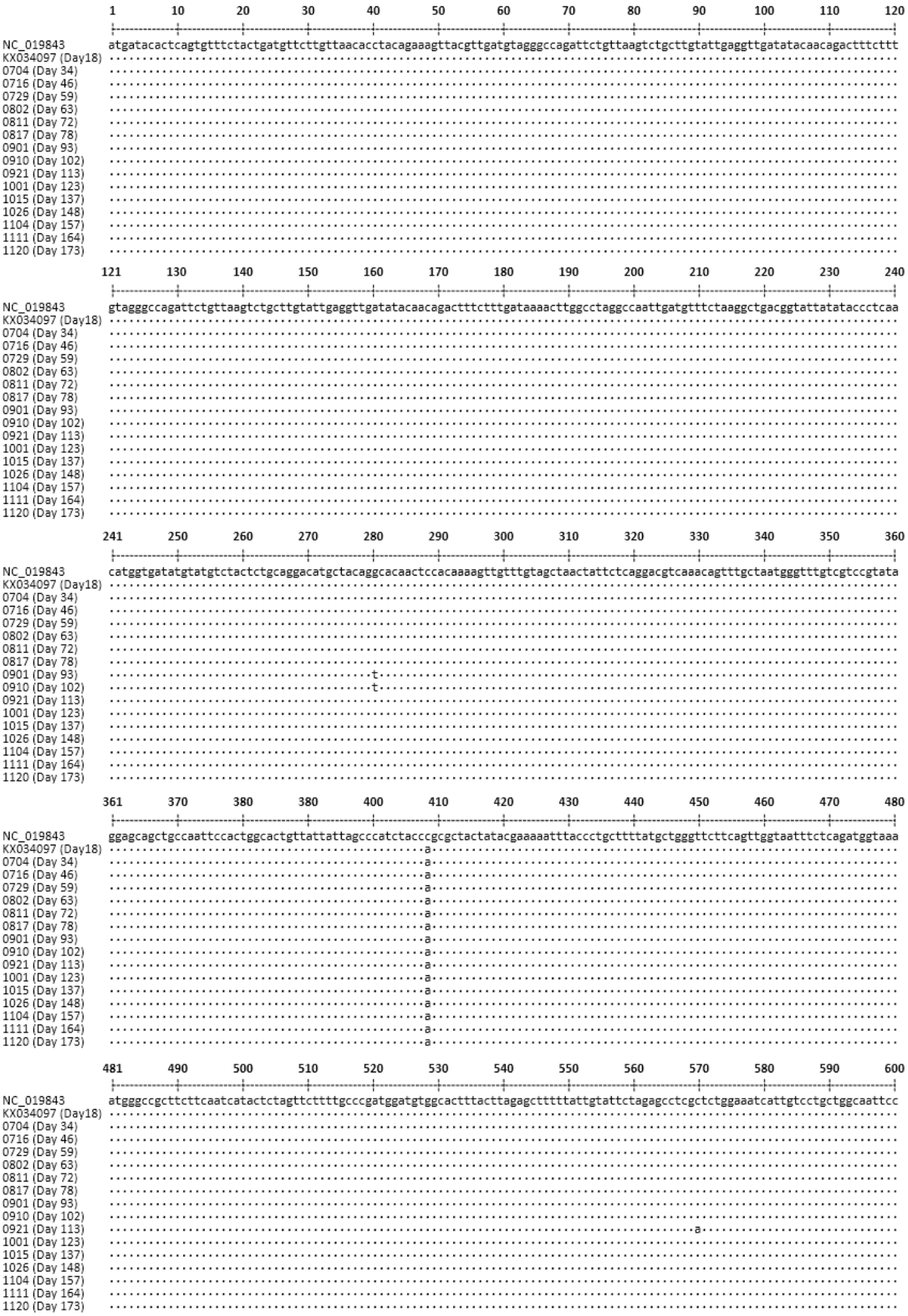
PCR product for S protein was obtained from respiratory samples directly and checked and purified on an agarose gel. Next-Generation Sequencing (NGS) library was constructed using TruSeq Nano DNA HT Library Prep kit (Illumina, CA, USA), and NGS was performed using MiSeq 300 cycles v2 reagent (Illumina, CA, USA). The NGS data were aligned to MERS-CoV, NC_019843, used for Binary Sequence Alignment/Map (BAM) file generation. We compared sequences from samples

obtained at different times and sequence of MERS-CoV/KOR/Seoul/080-3-2015 strain (GenBank accession No. KX034097), which was obtained by direct sequencing of the same patient on day 17 after symptom onset [3].

Reference

1. Park WB, Kwon NJ, Choe PG, et al. Isolation of Middle East Respiratory Syndrome Coronavirus from a Patient of the 2015 Korean Outbreak. J Korean Med Sci **2016**; 31(2): 315-20.
2. Park WB, Poon LLM, Choi SJ, et al. Replicative virus shedding in the respiratory tract of patients with Middle East respiratory syndrome coronavirus infection. Int J Infect Dis **2018**; 72: 8-10.
3. Park D, Huh HJ, Kim YJ, et al. Analysis of intrapatient heterogeneity uncovers the microevolution of Middle East respiratory syndrome coronavirus. Cold Spring Harbor molecular case studies **2016**; 2(6): a001214.

Figure S1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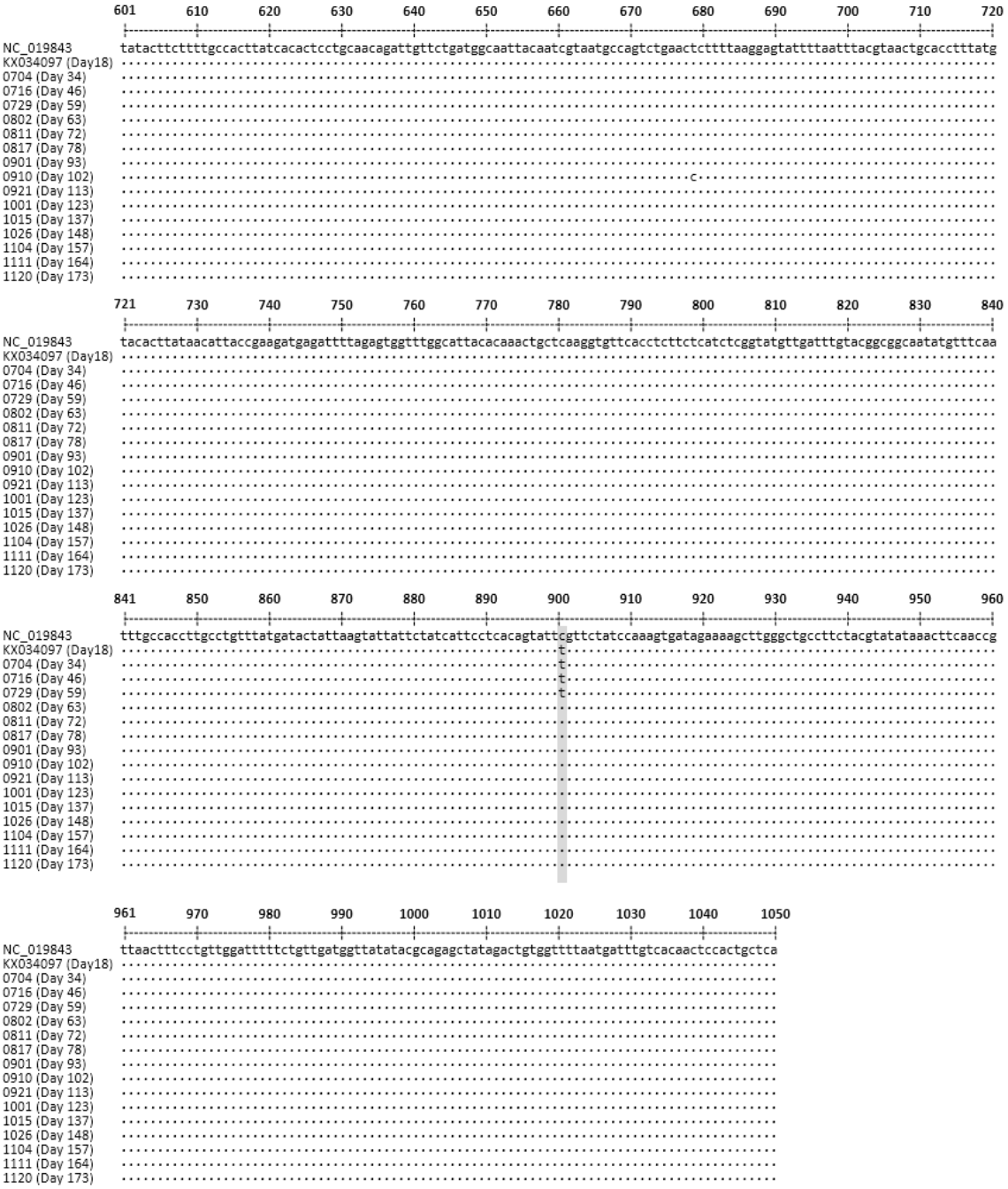
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